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## (54) Title: PROTEINS, GENES AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF SCHIZOPHRENIA

(57) Abstract: The present invention provides methods and compositions for screening, diagnosis and prognosis of Schizophrenia, as well as for monitoring the effectiveness of Schizophrenia treatment, identifying patients most likely to respond to a particular therapeutic modality or agent for drug development. Schizophrenia-Associated Proteins (SAPs), detectable by two-dimensional electrophoresis of cerebrospinal fluid, serum or plasma are described. The invention further provides Schizophrenia-Associated Protein Isoforms (SPIs) detectable in cerebrospinal fluid, serum or plasma, preparations comprising SPIs, antibodies immunospecific for SPIs, and kits comprising the aforesaid.

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candidate genes, such as the dopamine D3 receptor gene (Murphy et al, *J Mol Neurosci* (1996) 7:147-57). However, volumetric losses in the cerebral hemisphere and as well as changes in physiological and neuropsychological performance deficits such as a decreased prefrontal regional cerebral blood flow in the same twin studies suggest a significant contribution of nonheritable factors to the pathogenesis of Schizophrenia (Goldberg *Psychiatry Res.* (1994) 55:51-61).

Although genetics and genotyping may help to define the heritable risk for Schizophrenia, the utility for diagnosis, prognosis and treatment of Schizophrenia may be considerably less. Furthermore, no CNS tissue necessary for any gene expression analysis can be obtained for a living patient under normal circumstances. Proteomic approaches appear most suitable for a molecular dissection of such disease phenotypes in the central nervous system (CNS). The entire CNS is largely inaccessible to meaningful mRNA expression-based analyses of primary human material, since post mortem delays in primary human brain tissue affects mRNAs more readily than proteins (Edgar et al, *Molecular Psychiatry* (1999) 4:173-177). Given that the CSF bathes the brain, changes in its protein composition may reveal alterations in CNS protein expression patterns causatively or diagnostically linked to the disease. Reasonable amounts of disease associated proteins (DAPs) are secreted or released into body fluids by diseased tissue in the living patient at the onset and/or during progression of the disease. In many cases these alterations will be independent of the genetic makeup of the individual and rather directly related to a set of molecular and cellular alterations contribution to the pathogenic phenotype (Carpenter *J Psychiatr Res* (1998) 32:191-5).

Currently diagnosis of Schizophrenia remains clinically based on the presence of certain types of hallucinations, delusions and thought disorders (Andreasen *Lancet* (1995) 346:477-481). It is made on the basis of a careful clinical interview and mental status examination according to international established manuals, in particular the DSM-IV or ICD 10. The core clinical symptoms comprise formal thought disorders, delusions, hallucinations (also summarized as positive symptoms), and negative symptoms such as lack of drive and affect flattening. Neuroimaging techniques such as magnetic resonance imaging or positron emission tomography show subtle changes of the frontal and temporal lobes and the basal ganglia (Buchsbaum, *Schiz. Bull.* (1990) 16:379-389) in the majority of patients. Since these alterations are of little value for the diagnosis, treatment, or prognosis of the disorder in individual patients the role of the neuroimaging techniques mentioned above is by and large restricted to the exclusion of other conditions which may be accompanied by schizophrenic symptoms such as brain tumors, hemorrhages, or – in combination with chemical parameters obtained in CSF-samples – infections of the central nervous system.

Neuroleptic agents are essential for the treatment of Schizophrenia. While typical neuroleptics effect primarily the dopaminergic system, newer atypical neuroleptics also affect

serotonergic synapses. In general, the latter have greater effects on negative symptoms and cause less extrapyramidal side effects than typical neuroleptic compounds. It is generally accepted that early and continuous neuroleptic treatment may improve the outcome of the disorder. Nevertheless, regardless of the particular drug used, neuroleptic treatment is still considered to be solely symptomatic and does not inhibit the causes of the disorder.

Currently Schizophrenia has no objective biochemical markers useful for diagnosis and prognosis in living patients. Many CNS pathologies involve increased neuronal loss and such neuronal loss or impaired synaptogenesis may result in disease associated alterations of neuronal and CSF proteins. Synaptic pathologies have been implicated in Schizophrenia (Heinonen et al, *Neuroscience* (1995) 64:375-384; Bencs *Schiz. Bull.* (1993) 19:537-549). Consequently, it is not surprising that changes in synaptic proteins such as SNAP 25 (Thompson et al, *Neuropharmacology* (1999) 21:717-22), neureitin (Sharma et al, *Am J Psychiatry* (1997) 154:1019-21) and N-CAM (Vawter et al, *Schizophr Res* (1998) 34:123-31) have been detected in CSF of Schizophrenia patients. N-CAM levels are altered in affected twins and not in healthy siblings (Polkarik et al, *Brain Res* (1997) 751:152-4) suggesting they may be directly linked to the pathogenesis of Schizophrenia. Such DAPs may provide important insights into disease pathology and opportunities for better diagnosis and treatment strategies. However, these changes may also occur in other diseases, such as the elevation of -2-haptoglobin in Schizophrenia and Alzheimer's disease (Johnson et al, *Applied and Theoretical Electrophoresis* (1992) 3:47-53) and elevated SNAF-25 levels in Schizophrenia and bipolar patients (Thompson op. cit). Therefore, the specificity and the sensitivity of distinguishing individual neurological disorders as well as acute and chronic CNS disease may require the selection of a repertoire of DAPs rather than an individual protein.

Due to the high rates at which other disorders co-occur with Schizophrenia, the time consuming nature of existing, largely inadequate, tests and their expense it would be highly desirable to measure a substance or substances in samples of cerebrospinal fluid (CSF), blood or urine that would lead to a positive diagnosis of Schizophrenia or that would help to exclude Schizophrenia from the differential diagnosis.

Therefore, a need exists to identify DAPs as sensitive and specific biomarkers for the diagnosis of Schizophrenia in living subjects. Additionally, there is a clear need for new therapeutic agents for Schizophrenia that work quickly, potently, specifically, and with fewer side effects.

The present invention provides methods and compositions for clinical screening, diagnosis, prognosis, therapy and prophylaxis of Schizophrenia, for monitoring the

PROTEINS, GENES AND THEIR USE FOR  
DIAGNOSIS AND TREATMENT OF SCHIZOPHRENIA

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

## 1. INTRODUCTION

The present invention relates to the identification of proteins and protein isoforms that are associated with Schizophrenia and its onset and development, and of genes encoding the same, and to their use for e.g., clinical screening, diagnosis, prognosis, therapy and prophylaxis, as well as for drug screening and drug development.

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## 2. BACKGROUND OF THE INVENTION

In the majority of psychiatric disorders, little is known about a link between changes at a cellular and/or molecular level and nervous system structure and function. The paucity of detectable neuralgic defects distinguishes neuropsychiatric disorders such as Schizophrenia from neurological disorders, where manifestations of anatomical and biochemical changes have been identified in many cases. Consequently the identification and characterization of cellular and/or molecular causative defects and neuropathologies are necessary for improved treatment of neuropsychiatric disorders.

Schizophrenia is characterized by episodes of positive symptoms such as delusions, hallucinations, paranoia and psychosis and/or negative symptoms such as flattened affect, impaired attention, social withdrawal, and cognitive impairments (Ban et al, *Psychiatr. Dev.* (1984) 2:179-199). It afflicts 1.1% of the U.S. population and imposes a disproportionately large economic burden due to long-term expenditures for hospitalization, treatment and rehabilitation, and lost productivity. Cost-of-illness studies have estimated that in 1990 the total economic burden of Schizophrenia in the US was \$32.5 billion. (*Rico J Clin Psychiatry* (1999) 60 Suppl 1 4-6). In the UK, the total discounted cost to society attributable to an annual cohort of newly-diagnosed patients with Schizophrenia over the first 5 years following diagnosis has been estimated at £-662 million (Guest and Cookson *Pharmacoeconomics* (1999) 15:597- 610). Co-morbid substance abuse disorders have emerged as one of the greatest obstacles to the effective treatment of persons with Schizophrenia. Estimates of the prevalence of such co-morbidity vary, but as many as half of persons with Schizophrenia may suffer from a co-morbid drug or alcohol disorder (Dixon *Schizophr Res* (1999) 35 Suppl:S 93-100). Effective treatments used early in the course of Schizophrenia can help reduce the costs associated with this illness.

The relative contribution of genetic and environmental factors to the disease etiology remain uncertain, although an increased prevalence of Schizophrenia has been demonstrated in family and twin studies (Kendler *Am. J. Psychiatry* (1983) 140:1413- 1425) and resulted in the identification of candidate chromosomes including chromosome 6 and 22 and several

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candidate genes, such as the dopamine D3 receptor gene (Murphy et al, *J Mol Neurosci* (1996) 7:147-57). However, volumetric losses in the cerebral hemisphere and as well as changes in physiological and neuropsychological performance deficits such as a decreased prefrontal regional cerebral blood flow in the same twin studies suggest a significant contribution of nonheritable factors to the pathogenesis of Schizophrenia (Goldberg *Psychiatry Res.* (1994) 55:51-61).

Although genetics and genotyping may help to define the heritable risk for Schizophrenia, the utility for diagnosis, prognosis and treatment of Schizophrenia may be considerably less. Furthermore, no CNS tissue necessary for any gene expression analysis can be obtained for a living patient under normal circumstances. Proteomic approaches appear most suitable for a molecular dissection of such disease phenotypes in the central nervous system (CNS). The entire CNS is largely inaccessible to meaningful mRNA expression-based analyses of primary human material, since post mortem delays in primary human brain tissue affects mRNAs more readily than proteins (Edgar et al, *Molecular Psychiatry* (1999) 4:173-177). Given that the CSF bathes the brain, changes in its protein composition may reveal alterations in CNS protein expression patterns causatively or diagnostically linked to the disease. Reasonable amounts of disease associated proteins (DAPs) are secreted or released into body fluids by diseased tissue in the living patient at the onset and/or during progression of the disease. In many cases these alterations will be independent of the genetic makeup of the individual and rather directly related to a set of molecular and cellular alterations contribution to the pathogenic phenotype (Carpenter *J Psychiatr Res* (1998) 32:191-5).

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Due to the high rates at which other disorders co-occur with Schizophrenia, the time consuming nature of existing, largely inadequate, tests and their expense it would be highly desirable to measure a substance or substances in samples of cerebrospinal fluid (CSF), blood or urine that would lead to a positive diagnosis of Schizophrenia or that would help to exclude Schizophrenia from the differential diagnosis.

Therefore, a need exists to identify DAPs as sensitive and specific biomarkers for the diagnosis of Schizophrenia in living subjects. Additionally, there is a clear need for new therapeutic agents for Schizophrenia that work quickly, potently, specifically, and with fewer side effects.

The present invention provides methods and compositions for clinical screening, diagnosis, prognosis, therapy and prophylaxis of Schizophrenia, for monitoring the

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effectiveness of Schizophrenia treatment, for selecting participants in clinical trials, for identifying patients most likely to respond to a particular therapeutic treatment and for screening and development of drugs for treatment of Schizophrenia. A first aspect of the invention provides methods for diagnosis of Schizophrenia that comprise analyzing a sample of cerebrospinal fluid (CSF) by two-dimensional electrophoresis to detect the presence or level of at least one Schizophrenia-Associated Feature (SF), e.g., one or more of the SFs disclosed herein or any combination thereof. These methods are also suitable for clinical screening, prognosis, monitoring the results of therapy, identifying patients most likely to respond to a particular therapeutic treatment, for drug screening and development, and identification of new targets for drug treatment.

A second aspect of the invention provides methods for diagnosis of Schizophrenia that comprise detecting in a sample of CSF the presence or level of at least one Schizophrenia-Associated Protein Isoform (SPI), e.g., one or more of the SPIs disclosed herein or any combination thereof. These methods are also suitable for clinical screening, prognosis, monitoring the results of therapy, identifying patients most likely to respond to a particular therapeutic treatment, drug screening and development, and identification of new targets for drug treatment.

A third aspect of the invention provides antibodies, e.g. monoclonal and polyclonal antibodies capable of immunospecific binding to an SPI, e.g., an SPI disclosed herein.

A fourth aspect of the invention provides a preparation comprising an isolated SPI, i.e., an SPI free from proteins or protein isoforms having a significantly different isoelectric point or a significantly different apparent molecular weight from the SPI.

A fifth aspect of the invention provides methods of treating Schizophrenia, comprising administering to a subject a therapeutically effective amount of an agent that modulates (e.g., upregulates or downregulates) the expression or activity (e.g. enzymatic or binding activity), or both, of an SPI in subjects having Schizophrenia, in order to prevent or delay the onset or development of Schizophrenia, to prevent or delay the progression of Schizophrenia, or to ameliorate the symptoms of Schizophrenia.

A sixth aspect of the invention provides methods of screening for agents that modulate (e.g., upregulate or downregulate) a characteristic of, e.g., the expression or the enzymatic or binding activity, of an SPI, an SPI analog, or an SPI-related polypeptide.

### 3.1. Definitions

The term "SPI analog" as used herein refers to a polypeptide that possesses a similar or identical function as an SPI but need not necessarily comprise an amino acid sequence that is similar or identical to the amino acid sequence of the SPI, or possess a structure that is similar or identical to that of the SPI. As used herein, an amino acid sequence of a

polypeptide is "similar" to that of an SPI if it satisfies at least one of the following criteria: (a) the polypeptide has an amino acid sequence that is at least 30% (more preferably, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of the SPI; (b) the polypeptide is encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding at least 5 amino acid residues (more preferably, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino acid residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, or at least 150 amino acid residues) of the SPI; or (c) the polypeptide is encoded by a nucleotide sequence that is at least 30% (more preferably, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99%) identical to the nucleotide sequence encoding the SPI. As used herein, a polypeptide with "similar structure" to that of an SPI refers to a polypeptide that has a similar secondary, tertiary or quaternary structure as that of the SPI. The structure of a polypeptide can be determined by methods known to those skilled in the art, including but not limited to, X-ray crystallography, nuclear magnetic resonance, and crystallographic electron microscopy.

The term "SPI fusion protein" as used herein refers to a polypeptide that comprises (i) an amino acid sequence of an SPI, an SPI fragment, an SPI-related polypeptide or a fragment of an SPI-related polypeptide and (ii) an amino acid sequence of a heterologous polypeptide (i.e., a non-SPI, non-SPI fragment or non-SPI-related polypeptide).

The term "SPI homolog" as used herein refers to a polypeptide that comprises an amino acid sequence similar to that of an SPI but does not necessarily possess a similar or identical function to that of the SPI.

The term "SPI ortholog" as used herein refers to a non-human polypeptide that (i) comprises an amino acid sequence similar to that of an SPI and (ii) possesses a similar or identical function to that of the SPI.

The term "SPI-related polypeptide" as used herein refers to an SPI homolog, an SPI analog, an isoform of SPI, an SPI ortholog, or any combination thereof.

The term "derivative" as used herein refers to a polypeptide that comprises an amino acid sequence of a second polypeptide which has been altered by the introduction of amino acid residue substitutions, deletions or additions. The derivative polypeptide possess a similar or identical function as the second polypeptide.

The term "fragment" as used herein refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 amino acid residues (preferably, at least 10 amino acid

residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, at least 150 amino acid residues, at least 175 amino acid residues, at least 200 amino acid residues, or at least 250 amino acid residues) of the amino acid sequence of a second polypeptide. The fragment of an SPI may or may not possess a functional activity of the second polypeptide.

The term "fold change" includes "fold increase" and "fold decrease" and refers to the relative increase or decrease in abundance of an SF or the relative increase or decrease in expression or activity of a polypeptide (e.g., an SPI) in a first sample or sample set compared to a second sample (or sample set). An SF or polypeptide fold change may be measured by any technique known to those of skill in the art, however the observed increase or decrease will vary depending upon the technique used. Preferably, fold change is determined herein as described in the Examples infra.

The term "isoform" as used herein refers to variants of a polypeptide that are encoded by the same gene, but that differ in their pI or MW, or both. Such isoforms can differ in their amino acid composition (e.g., as a result of alternative splicing or limited proteolysis) and in addition, or in the alternative, may arise from differential post-translational modification (e.g., glycosylation, acylation, phosphorylation). As used herein, the term "isoform" also refers to a protein that exists in only a single form, i.e., it is not expressed as several variants.

The term "modulate" when used herein in reference to expression or activity of an SPI or an SPI-related polypeptide refers to the upregulation or downregulation of the expression or activity of the SPI or an SPI-related polypeptide. Based on the present disclosure, such modulation can be determined by assays known to those of skill in the art or described herein.

The percent identity of two amino acid sequences or of two nucleic acid sequences is determined by aligning the sequences for optimal comparison purposes (e.g., gaps can be introduced in the first sequence for best alignment with the sequence) and comparing the amino acid residues or nucleotides at corresponding positions. The "best alignment" is an alignment of two sequences which results in the highest percent identity. The percent identity is determined by the number of identical amino acid residues or nucleotides in the sequences being compared (i.e., % identity = # of identical positions/total # of positions x 100).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those of skill in the art. An example of a mathematical algorithm for comparing two sequences is the algorithm of Karlin and Altschul *Proc. Natl. Acad. Sci. USA* (1990) 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. The NBLAST and XBLAST programs of Altschul

et al, *J. Mol. Biol.* (1990) 215:403-410 have incorporated such an algorithm. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al, *Nucleic Acids Res.* (1997) 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

Another example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). The ALIGN program (version 2.0) which is part of the GCG sequence alignment software package has incorporated such an algorithm. Other algorithms for sequence analysis known in the art include ADVANCE and ADAM as described in Torellis and Robotti *Comput. Appl. Biosci.* (1994) 10:3-5; and FASTA described in Pearson and Lipman *Proc. Natl. Acad. Sci. USA* (1988) 85:2444-8. Within FASTA, knp is a control option that sets the sensitivity and speed of the search.

### 4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is an image obtained from 2-dimensional electrophoresis of human CSF, which has been annotated to identify twelve landmark features, designated CSF1 to CSF12.

Figure 2 comprises amino acid sequences of SPI-206 (Figure 2A) and the nucleic acid sequence encoding the amino acid sequence of Figure 2A (Figure 2B). Peptides of SPI-206 identified by mass spectrometry are underlined in the sequence of Figure 2A, and the amino acid sequences determined by mass spectrometry are highlighted.

Figure 3 shows tissue distribution of SPI-206 mRNA. Levels of mRNA in samples of normal tissue were quantified by real time RT-PCR. The mRNA levels are expressed as the number of copies per nanogram cDNA. Note the 25 times difference in scale between the graph containing brain-related samples, and the graph containing body samples.

Figure 4 comprises amino acid sequences of SPI-238 and SPI-240 (Figure 2A) and the nucleic acid sequence encoding the amino acid sequence of Figure 2A (Figure 2B). Peptides of SPI-238 and SPI-240 identified by mass spectrometry are underlined in the sequence of Figure 2A, and the amino acid sequences determined by mass spectrometry are highlighted.

Figure 5 shows tissue distribution of SPI-238 and SPI-240 mRNA. Levels of mRNA in samples of normal tissue were quantified by real time RT-PCR. The mRNA levels are expressed as the number of copies per nanogram cDNA. Note the 25 times difference in scale between the graph containing brain-related samples, and the graph containing body samples.

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##### 5. DETAILED DESCRIPTION OF THE INVENTION

The invention described in detail below provides methods and compositions for clinical screening, diagnosis and prognosis of Schizophrenia in a mammalian subject for identifying patients most likely to respond to a particular therapeutic treatment, for monitoring the results of Schizophrenia therapy, for drug screening and drug development. The invention also encompasses the administration of therapeutic compositions to a mammalian subject to treat or prevent Schizophrenia. The mammalian subject may be a non-human mammal, but is preferably human more preferably a human adult, i.e. a human subject at least 21 (more preferably at least 35, at least 50, at least 60, at least 70, or at least 80) years old. For clarity of disclosure, and not by way of limitation, the invention will be described with respect to the analysis of CSF samples. However, as one skilled in the art will appreciate, the assays and techniques described below can be applied to other types of samples, including a body fluid (e.g. blood, serum, plasma, saliva or urine), a tissue sample from a subject at risk of having or developing Schizophrenia (e.g. a biopsy such as a brain biopsy) or homogenate thereof. The methods and compositions of the present invention are useful for screening, diagnosis and prognosis of a living subject, but may also be used for postmortem diagnosis in a subject, for example, to identify family members of the subject who are at risk of developing the same disease.

As used herein, cerebrospinal fluid (CSF) refers to the fluid that surrounds the bulk of the central nervous system, as described in *Physiological Basis of Medical Practice* (J.B. West, ed., Williams and Wilkins, Baltimore, MD 1985). CSF includes ventricular CSF and lumbar CSF. As used herein, the term "serum" refers to the supernatant fluid produced by clotting and centrifugal sedimentation of a blood sample. As used herein, the term "plasma" refers to the supernatant fluid produced by inhibition of clotting (for example, by citrate or EDTA) and centrifugal sedimentation of a blood sample. The term "blood" as used herein includes serum and plasma.

##### 5.1 Schizophrenia-Associated Features (SFs)

In one aspect of the invention, two-dimensional electrophoresis is used to analyze CSF from a subject, preferably a living subject, in order to detect or quantify the expression of one or more Schizophrenia-Associated Features (SFs) for screening, prevention or diagnosis of Schizophrenia, to determine the prognosis of a subject having Schizophrenia, to monitor

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A more highly preferred scanner is the Apollo 2 scanner (Oxford Glycosciences, Oxford, UK), which is a modified version of the above described scanner. In the Apollo 2 scanner, the gel is transported through the scanner on a precision lead-screw drive system. This is preferable to laying the glass plate on the belt-driven system that is described in the Basiji thesis, as it provides a reproducible means of accurately transporting the gel past the imaging optics.

In the Apollo 2 scanner, the gel is secured against three alignment stops that rigidly hold the glass plate in a known position. By doing this in conjunction with the above precision transport system, the absolute position of the gel can be predicted and recorded. This ensures that co-ordinates of each feature on the gel can be determined more accurately and communicated, if desired, to a cutting robot for excision of the feature. In the Apollo 2 scanner, the carrier that holds the gel has four integral fluorescent markers for use to correct the image geometry. These markers are a quality control feature that confirms that the scanning has been performed correctly.

In comparison to the scanner described in the Basiji thesis, the optical components of the Apollo 2 scanner have been inverted. In the Apollo 2 scanner, the laser, mirror, waveguide and other optical components are above the glass plate being scanned. The scanner described in the Basiji thesis has these components underneath. In the Apollo 2 scanner, the glass plate is mounted onto the scanner gel side down, so that the optical path remains through the glass plate. By doing this, any particles of gel that may break away from the glass plate will fall onto the base of the instrument rather than into the optics. This does not affect the functionality of the system, but increases its reliability.

Still more preferred is the Apollo 3 scanner, in which the signal output is digitized to the full 16-bit data without any peak saturation or without square root encoding of the signal. A compensation algorithm has also been applied to correct for any variation in detection sensitivity along the path of the scanning beam. This variation is due to anomalies in the optics and differences in collection efficiency across the waveguide. A calibration is performed using a perspex plate with an even fluorescence throughout. The data received from a scan of this plate are used to determine the multiplication factors needed to increase the signal from each pixel level to a target level. These factors are then used in subsequent scans of gels to remove any internal optical variations.

As used herein, the term "feature" refers to a spot detected in a 2D gel, and the term "Schizophrenia-Associated Feature" (SF) refers to a feature that is differentially present in a sample (e.g. a sample of CSF) from a subject having Schizophrenia compared with a sample (e.g. a sample of CSF) from a subject free from Schizophrenia. As used herein, a feature (or a protein isoform of SPI, as defined *infra*) is "differentially present" in a first sample with respect to a second sample when a method for detecting the feature, isoform or SPI (e.g., 2D

progression of Schizophrenia, to monitor the effectiveness of Schizophrenia therapy, for identifying patients most likely to respond to a particular therapeutic treatment, or for drug development. As used herein, "two-dimensional electrophoresis" (2D-electrophoresis) means a technique comprising isoelectric focusing, followed by denaturing electrophoresis; this generates a two-dimensional gel (2D-gel) containing a plurality of separated proteins. Preferably, the step of denaturing electrophoresis uses polyacrylamide electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). Especially preferred are the highly accurate and automatable methods and apparatus ("the Preferred Technology") described in International Application No. 97GB3307 (published, as WO 98/23950) and in U.S. Application No. 08/980,574, both filed December 1, 1997, each of which is incorporated herein by reference in its entirety with particular reference to the protocol at pages 23-35. Briefly, the Preferred Technology provides efficient, computer-assisted methods and apparatus for identifying, selecting and characterizing biomolecules (e.g. proteins, including glycoproteins) in a biological sample. A two-dimensional array is generated by separating biomolecules on a two-dimensional gel according to their electrophoretic mobility and isoelectric point. A computer-generated digital profile of the array is generated, representing the identity, apparent molecular weight, isoelectric point, and relative abundance of a plurality of biomolecules detected in the two-dimensional array, thereby permitting computer-mediated comparison of profiles from multiple biological samples, as well as computer aided excision of separated proteins of interest.

A preferred scanner for detecting fluorescently labelled proteins is described in WO 96/36882 and in the Ph.D. thesis of David A. Basiji, entitled "Development of a High-throughput Fluorescence Scanner Employing Internal Reflection Optics and Phase-sensitive Detection (Total Internal Reflection Electrophoresis)", University of Washington (1997), Volume 58/12-B of Dissertation Abstracts International, page 6686, the contents of each of which are incorporated herein by reference. These documents describe an image scanner designed specifically for automated, integrated operation at high speeds. The scanner can image gels that have been stained with fluorescent dyes or silver stains, as well as storage phosphor screens. The Basiji thesis provides a phase-sensitive detection system for discriminating modulated fluorescence from baseline noise due to laser scatter or homogeneous fluorescence, but the scanner can also be operated in a non-phase-sensitive mode. This phase-sensitive detection capability would increase the sensitivity of the instrument by an order of magnitude or more compared to conventional fluorescence imaging systems. The increased sensitivity would reduce the sample-preparation load on the upstream instruments while the enhanced image quality simplifies image analysis downstream in the process.

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electrophoresis or an immunoassay) gives a different signal when applied to the first and second samples. A feature, isoform or SPI is "increased" in the first sample with respect to the second if the method of detection indicates that the feature, isoform or SPI is more abundant in the first sample than in the second sample, or if the feature, isoform or SPI is detectable in the first sample and undetectable in the second sample. Conversely, a feature, isoform or SPI is "decreased" in the first sample with respect to the second if the method of detection indicates that the feature, isoform or SPI is less abundant in the first sample than in the second sample or if the feature, isoform or SPI is undetectable in the first sample and detectable in the second sample.

Preferably, the relative abundance of a feature in two samples is determined in two steps. First, the signal obtained upon detecting the feature in a sample is normalized by reference to a suitable background parameter, e.g., (a) to the total protein in the sample being analyzed (e.g., total protein loaded onto a gel); (b) to an Expression Reference Feature (ERF) i.e., a feature whose abundance is invariant, within the limits of variability of the Preferred Technology, in the population of subjects being examined, e.g. the ERFs disclosed below, or (c) more preferably to the total signal detected from all proteins in the sample.

Secondly, the normalized signal for the feature in one sample or sample set is compared with the normalized signal for the same feature in another sample or sample set in order to identify features that are "differentially present" in the first sample (or sample set) with respect to the second.

The SFs disclosed herein have been identified by comparing CSF samples from subjects having Schizophrenia against CSF samples from subjects free from Schizophrenia. Subjects free from Schizophrenia include subjects with no known disease or condition (normal subjects) and subjects with diseases (including neurological and neurodegenerative diseases) other than Schizophrenia.

Two groups of SFs have been identified through the methods and apparatus of the Preferred Technology. The first group consists of SFs that are decreased in the CSF of subjects having Schizophrenia as compared with the CSF of subjects free from Schizophrenia. These SFs can be described by apparent molecular weight (MW) and isoelectric point (pI) as provided in Table I.

Table I. SFs Decreased in CSF of Subjects Having Schizophrenia

SF#	pI	MW (Da)	Fold Decrease	Rank Sum P-Value
SF-14	6.24	102603	44.24	
SF-16	4.73	26954	12.99	
SF-17 ..	4.89	18534	8.71	

SF-18	6.04	43920	6.38	
SF-19	8.99	21601	7.11	
SF-20	4.25	64918	7.51	
SF-21	7.10	10885	6.07	
SF-22	9.58	20268	6.32	
SF-23	9.81	14171	6.52	
SF-24	4.81	12637	6.62	0.03689
SF-25	4.19	71670	5.78	
SF-26	7.17	47823	4.79	
SF-27	8.14	13783	4.90	
SF-28	9.25	12001	3.99	
SF-29	8.89	11749	3.71	
SF-30	4.52	109372	4.19	
SF-31	5.43	112518	4.07	
SF-32	5.43	48238	3.27	0.01219
SF-33	4.25	106909	3.85	
SF-34	9.83	10120	3.29	
SF-35	5.03	36795	3.48	
SF-36	9.58	21021	3.44	
SF-37	6.08	93159	2.94	0.03671
SF-38	5.67	48092	2.37	0.01219
SF-39	4.67	14570	2.94	0.01996
SF-40	6.93	27331	2.11	0.01219
SF-41	5.19	50178	2.23	0.01219
SF-42	5.98	90092	2.04	0.03671
SF-43	5.43	49573	2.13	0.03577
SF-44	8.16	24182	1.88	0.01219
SF-45	5.30	49423	1.90	0.01219
SF-46	7.39	68161	1.73	0.03615
SF-47	4.86	38741	1.94	0.03671
SF-48	5.11	35613	1.74	0.01219
SF-49	5.90	23795	1.56	0.02157
SF-51	7.10	23117	1.43	0.02157
SF-52	6.00	49723	1.78	0.02157
SF-53	4.72	20882	1.60	0.01193
SF-55	4.94	59288	1.69	0.03671
SF-56	5.04	57690	1.57	0.01219
SF-57	6.36	20134	1.30	0.02118
SF-58	7.20	19285	1.44	0.01945
SF-368	6.18	105482	31.19	
SF-369	4.39	62654	26.06	

SF-370	7.71	57865	14.56	
SF-371	7.27	26683	11.95	
SF-372	6.58	14769	11.27	
SF-373	5.96	99056	10.85	
SF-374	5.00	161367	8.83	
SF-375	7.38	38741	7.65	
SF-376	5.42	18290	7.30	
SF-377	6.18	187641	7.18	
SF-378	6.45	60068	6.40	
SF-379	5.12	15174	6.21	
SF-380	9.83	39766	5.95	
SF-381	4.70	19478	5.65	
SF-382	8.54	54625	5.05	
SF-383	7.49	52637	4.74	
SF-384	6.27	186027	4.29	
SF-385	5.99	147226	4.20	
SF-386	6.94	70146	4.02	
SF-387	6.58	93680	3.79	
SF-388	5.89	102725	3.53	
SF-389	5.19	25665	3.45	
SF-390	6.30	186832	3.44	
SF-391	4.53	35202	3.32	
SF-392	4.99	21951	3.28	
SF-393	6.78	24182	3.20	
SF-394	6.45	16614	3.18	
SF-395	4.51	12420	3.11	
SF-396	5.56	23599	3.04	
SF-397	9.39	11427	2.99	
SF-398	6.32	22090	2.97	
SF-399	8.17	12814	2.93	
SF-400	7.50	20201	2.92	
SF-401	5.09	11621	2.89	
SF-402	6.03	13175	2.88	
SF-403	4.80	49063	2.15	
SF-404	4.50	32266	2.12	0.03734
SF-405	5.89	60151	1.90	
SF-406	4.91	38741	1.82	0.03671
SF-407	9.35	13879	1.78	
SF-408	7.20	37524	1.74	0.02157
SF-409	4.78	136566	1.69	0.01219
SF-410	5.13	65925	1.61	0.03689

SF-411	9.55	18969	1.54	
SF-412	4.62	36556	1.50	
SF-413	4.96	182153	1.41	
SF-414	5.03	65526	1.38	0.02157
SF-415	5.12	121995	1.33	
SF-416	4.99	58394	1.32	0.03671
SF-417	6.01	21999	1.27	
SF-418	9.54	26377	1.19	
SF-419	4.85	52993	1.18	
SF-420	4.63	27331	1.18	
SF-421	4.86	153822	1.16	
SF-422	5.84	65594	1.08	
SF-423	5.43	143548	1.04	

SF-94	4.26	113057	19.74	
SF-95	5.84	39743	13.92	
SF-96	6.05	63583	14.25	
SF-97	7.21	50321	13.81	
SF-98	5.87	45227	10.44	
SF-99	5.85	12549	11.10	
SF-100	6.39	14220	8.00	
SF-101	9.28	51958	10.65	
SF-102	6.56	14133	13.04	
SF-103	5.81	87306	7.41	
SF-104	7.68	130651	7.24	
SF-105	4.99	12018	10.36	
SF-106	6.81	12593	8.39	
SF-107	5.72	26522	8.58	
SF-108	6.12	15430	9.33	
SF-109	9.01	14280	7.99	
SF-110	5.74	111425	8.18	
SF-111	7.92	12182	10.87	
SF-112	5.79	14576	12.88	
SF-113	7.64	130785	5.91	
SF-114	4.47	13840	10.76	
SF-115	4.28	39080	9.55	
SF-116	6.85	30358	6.43	
SF-117	9.81	49898	7.08	
SF-118	6.86	50636	7.01	
SF-119	9.09	55966	10.76	
SF-120	6.26	12963	7.51	
SF-123	5.56	28440	6.57	0.01996
SF-124	6.89	19285	5.78	
SF-125	6.48	68087	7.59	
SF-126	6.19	46232	6.83	
SF-130	6.72	18040	3.84	
SF-131	7.97	64150	6.89	0.03577
SF-132	4.82	104557	7.57	
SF-135	7.23	31838	9.87	
SF-136	5.79	113516	6.37	
SF-137	5.83	13847	8.77	
SF-139	4.75	11568	6.66	
SF-141	6.45	137832	6.74	
SF-142	4.81	120492	5.94	
SF-143	7.48	43043	6.51	0.03689

Table II. SFs Increased in CSF of Subjects Having Schizophrenia

SF#.	pI	MW (Da)	Fold Increase	Rank Sum P-Value
SF-80	4.65	10120	50.81	
SF-81	5.39	28439	30.63	
SF-82	9.74	56994	26.22	
SF-83	7.65	61670	22.83	
SF-84	6.54	13783	22.09	
SF-85	6.60	14652	20.33	
SF-86	7.01	44684	19.00	
SF-87	5.37	29604	18.85	
SF-88	6.60	57865	18.41	
SF-89	4.78	14319	16.20	
SF-90	6.16	52513	15.76	
SF-91	5.64	14171	14.88	
SF-92	7.65	46181	12.52	
SF-93	6.61	11467	10.16	

SF-144	7.26	12594	7.16	
SF-147	7.38	125122	8.01	
SF-148	8.01	-41015	5.74	
SF-150	5.66	11503	6.00	
SF-151	6.28	30170	5.44	0.01996
SF-153	8.85	14369	5.11	0.03689
SF-154	6.39	12122	8.10	0.01996
SF-155	10.04	14024	8.39	
SF-157	4.85	26063	6.90	
SF-158	4.73	11509	6.46	
SF-159	5.03	18103	6.78	
SF-160	6.35	32266	4.30	0.03577
SF-161	6.55	12903	6.74	0.03689
SF-162	4.40	19285	5.81	0.03038
SF-163	8.26	14319	7.15	0.03689
SF-164	8.98	59466	6.46	0.03689
SF-165	6.45	20882	5.07	0.01219
SF-166	5.78	33716	6.36	0.03038
SF-167	5.17	15486	5.33	0.01996
SF-168	6.07	31433	3.53	0.03689
SF-169	5.78	29287	5.94	0.01219
SF-170	7.50	14319	4.08	0.03689
SF-171	6.69	24684	5.41	0.01996
SF-172	5.68	39422	5.09	0.01945
SF-173	6.39	44664	2.68	0.01996
SF-174	5.19	12080	3.27	0.03689
SF-175	5.78	179707	3.50	0.03689
SF-176	6.37	34096	3.96	0.01996
SF-177	4.46	48679	3.35	0.01996
SF-178	7.88	84540	3.59	0.01945
SF-179	6.05	30643	3.18	0.03689
SF-180	6.21	67544	4.12	0.01996
SF-181	6.29	80131	3.80	0.01996
SF-182	4.95	14570	4.65	0.03689
SF-183	6.67	- 38376	2.13	0.02940
SF-184	6.52	60192	3.21	0.03038
SF-185	7.48	59846	4.70	0.01996
SF-187	7.27	59466	3.57	0.01996
SF-188	7.01	40510	3.04	0.01219
SF-189	6.01	53953	2.58	0.03038
SF-190	4.91	70663	2.23	0.03734

SF-191	6.74	54791	4.40	0.02157
SF-194	7.03	55966	3.73	0.01996
SF-195	6.53	66326	2.79	0.03038
SF-196	5.52	178161	3.04	0.01945
SF-197	5.32	15381	4.88	0.01219
SF-198	7.73	15277	3.62	0.01219
SF-199	6.28	67135	3.75	0.01219
SF-200	6.03	135312	2.23	0.03038
SF-201	6.10	57615	2.41	0.02940
SF-202	5.13	42039	2.44	0.03689
SF-203	5.67	178932	2.78	0.03734
SF-204	5.49	38854	2.38	0.01996
SF-208	6.37	63376	2.48	0.01996
SF-209	6.53	10226	3.34	0.01996
SF-211	6.53	25861	2.94	0.03689
SF-212	6.48	179707	2.55	0.03671
SF-213	4.87	45882	2.47	0.02157
SF-215	5.55	178161	3.28	0.01219
SF-216	6.59	60374	2.38	0.03038
SF-217	6.03	17230	2.22	0.01219
SF-218	6.42	32454	1.63	0.03734
SF-219	6.56	20744	2.77	0.03689
SF-220	6.74	40716	1.79	0.01945
SF-221	6.88	100168	2.85	0.01945
SF-222	6.37	66932	3.08	0.01996
SF-226	4.81	50178	2.44	0.03734
SF-227	6.46	52673	2.13	0.01193
SF-228	6.97	14520	3.68	0.01219
SF-229	7.42	56138	3.58	0.01996
SF-230	4.31	63376	2.86	0.03038
SF-231	7.81	59828	2.82	0.01219
SF-232	7.31	64759	2.79	0.03689
SF-233	5.02	60026	2.47	0.03734
SF-235	4.49	18350	2.31	0.01996
SF-237	5.77	85533	1.63	0.03655
SF-238	5.77	19330	1.85	0.03671
SF-239	7.67	104514	2.62	0.03689
SF-242	5.48	11872	2.37	0.03577
SF-243	7.65	52513	2.07	0.01996
SF-244	6.65	12463	1.80	0.03734
SF-248	5.40	11986	2.14	0.03655

SF-249	6.18	178932	2.23	0.03655
SF-250	5.05	15381	3.03	0.01996
SF-255	7.03	155828	2.25	0.01193
SF-257	5.75	60558	2.60	0.01996
SF-258	5.06	49723	2.03	0.03689
SF-261	6.05	27854	1.69	0.03734
SF-262	6.72	57885	2.53	0.01996
SF-264	5.50	151166	2.30	0.01996
SF-265	6.90	166503	2.34	0.03689
SF-267	5.30	43920	2.02	0.03671
SF-268	7.22	155156	2.24	0.02157
SF-269	6.18	52038	1.98	0.03615
SF-271	5.06	13452	2.76	0.03038
SF-272	5.17	64933	1.57	0.01996
SF-273	6.09	67749	2.05	0.03671
SF-280	4.65	45728	1.82	0.03689
SF-282	4.88	31780	1.46	0.01193
SF-283	5.49	60558	2.17	0.03615
SF-286	4.99	61670	1.73	0.03689
SF-289	6.28	178161	2.02	0.02157
SF-291	7.14	32549	2.34	0.01996
SF-292	7.27	48075	1.89	0.01193
SF-293	9.24	35621	1.87	0.01996
SF-294	6.62	101681	2.20	0.01219
SF-296	6.52	175109	1.82	0.03615
SF-300	7.39	153822	1.81	0.02157
SF-301	7.14	95262	1.86	0.03577
SF-302	5.41	44684	1.70	0.02157
SF-303	6.88	40613	1.68	0.01167
SF-304	7.25	67622	1.81	0.02940
SF-306	5.72	100168	2.41	0.02000
SF-307	8.43	50638	1.83	0.01219
SF-309	5.28	72474	1.94	0.01219
SF-312	6.57	122917	1.59	0.02940
SF-317	5.59	43773	1.25	0.01945
SF-320	6.26	21818	1.84	0.01219
SF-321	6.72	101681	1.70	0.01193
SF-322	5.89	26797	1.52	0.01996
SF-324	6.20	43920	2.03	0.02157
SF-326	4.98	74524	1.82	0.02157
SF-327	4.40	16835	1.50	0.01219

SF-332	9.05	72071	2.19	0.03689
SF-333	4.50	47610	1.60	0.03671
SF-338	7.03	107446	1.97	0.01996
SF-340	5.03	46659	1.48	0.03671
SF-346	6.30	50790	1.75	0.01219
SF-349	7.16	39538	1.34	0.03615
SF-352	7.09	19543	1.62	0.03689
SF-358	5.67	68161	1.52	0.03038
SF-424	6.07	177393	1.02	
SF-425	8.69	61111	1.05	
SF-426	5.61	113833	1.05	
SF-427	5.53	91613	1.21	
SF-428	5.95	178932	1.22	
SF-429	5.56	34240	1.34	
SF-430	5.53	12527	1.37	
SF-431	6.07	62896	1.43	
SF-432	7.42	88485	1.44	
SF-433	4.81	32909	1.48	
SF-434	4.41	24762	1.49	
SF-435	9.82	57167	1.58	
SF-438	6.22	87429	1.60	
SF-437	7.49	118924	1.60	
SF-438	5.87	101769	1.61	
SF-439	7.15	11769	1.75	
SF-440	7.31	64933	1.77	
SF-441	6.88	42595	1.80	
SF-442	7.19	43883	1.81	
SF-443	4.24	39855	1.88	
SF-444	5.56	27604	2.00	
SF-445	6.65	40716	2.02	
SF-446	6.14	47484	2.04	
SF-447	7.85	45269	2.12	
SF-448	4.34	10961	2.12	
SF-449	5.32	16835	2.38	
SF-450	7.05	12377	2.44	
SF-451	9.28	17225	2.49	
SF-452	7.47	12814	2.61	
SF-453	8.07	11167	3.15	
SF-454	7.42	46424	3.18	
SF-455	5.73	15824	3.24	
SF-456	5.71	45728	3.32	

SF-457	5.76	45728	3.47
SF-458	6.65	13831	3.50
SF-459	9.81	29902	3.78
SF-460	8.76	11955	3.87
SF-461	6.29	18044	3.99
SF-462	5.00	31104	4.10
SF-463	5.95	29529	4.11
SF-464	8.19	27009	4.27
SF-465	6.48	21974	4.31
SF-466	6.28	48238	4.85
SF-467	5.88	12221	5.17
SF-468	5.58	73872	5.25
SF-469	4.44	100168	5.62
SF-470	5.70	79242	5.92
SF-471	4.88	15911	5.99
SF-472	7.51	24762	6.05
SF-473	6.89	64634	6.31
SF-474	6.22	17863	6.43
SF-475	5.68	73979	7.33
SF-476	4.24	65625	7.33
SF-477	8.04	55531	8.58
SF-478	6.80	32080	9.79
SF-479	5.19	39007	9.94
SF-480	6.06	34548	10.13
SF-481	8.44	41146	10.31
SF-482	9.70	57892	10.68
SF-483	6.58	44727	10.68
SF-484	5.62	38698	11.67
SF-485	7.09	42796	12.16
SF-486	5.31	39135	12.15
SF-487	7.28	34494	12.33
SF-488	6.23	167704	12.70
SF-489	6.35	31104	13.07
SF-490	7.08	84191	13.99
SF-491	6.31	43188	14.36
SF-492	6.28	41481	15.10
SF-493	5.27	11955	15.28
SF-494	6.69	39193	16.75
SF-495	5.75	11240	17.67
SF-496	6.82	109447	17.86
SF-497	9.60	51912	18.04

SF-498	10.65	11457	24.12
SF-499	7.68	12761	27.98
SF-500	5.84	13658	28.35
SF-501	7.88	24230	34.24
SF-502	4.23	39766	35.16

Where p values are given in Table II, the statistical technique used was the Wilcoxon Rank-Sum test as described in method (a) of Section 6.1.13 Statistical Analysis of the Profiles. Where no p value is reported, the method used to select these features was on the basis of fold change or qualitative presence or absence alone as described in methods (b) and (c) of Section 6.1.13 Statistical Analysis of the Profiles.

For any given SF, the signal obtained upon analyzing CSF from subjects having Schizophrenia relative to the signal obtained upon analyzing CSF from subjects free from Schizophrenia will depend upon the particular analytical protocol and detection technique that is used. Accordingly, the present invention contemplates that each laboratory will, based on the present description, establish a reference range for each SF in subjects free from Schizophrenia according to the analytical protocol and detection technique in use, as is conventional in the diagnostic art. Preferably, at least one control positive CSF sample from a subject known to have Schizophrenia or at least one control negative CSF sample from a subject known to be free from Schizophrenia (and more preferably both positive and negative control samples) are included in each batch of test samples analyzed. In one embodiment, the level of expression of a feature is determined relative to a background value, which is defined as the level of signal obtained from a proximal region of the image that (a) is equivalent in area to the particular feature in question; and (b) contains no discernable protein feature. The reference range, depending upon the method of detection used and the conditions under which detection is carried out, can include no feature or isoform present, or non-detectable levels of feature or isoform present. Proteins described by pI and MW provided in Tables I and II can be identified by searching 2D-PAGE databases with those pI and MW values. Examples of such databases are provided on the ExPASy Molecular Biology Server (<http://www.expasy.ch>) under the "SWISS-2DPAGE" section, and other databases are further referenced on this server. Such databases typically provide interactive 2D gel images for a given set of sample and preparation protocol, and the skilled artisan can obtain information relevant to a given feature by pointing and clicking the appropriate section of the image.

In a preferred embodiment, the signal associated with an SF in the CSF of a subject (e.g., a subject suspected of having or known to have Schizophrenia) is normalized with reference to one or more ERFs detected in the same 2D gel. As will be apparent to one of ordinary skill in the art, such ERFs may readily be determined by comparing different

samples using the Preferred Technology. Suitable ERFs include (but are not limited to) that described in the following table.

Table III. Expression Reference Features

ERP#	pI	MW (Da)
ERF-1	6.28	48238
ERF-2	4.28	26797

from a subject or subjects free from Schizophrenia (e.g., a control sample or a previously determined reference range) indicates the presence of Schizophrenia.

In another embodiment of the invention, CSF from a subject is analyzed by 2D electrophoresis for quantitative detection of one or more of the following SFs: SF-80, SF-81, SF-82, SF-83, SF-84, SF-85, SF-86, SF-87, SF-88, SF-89, SF-90, SF-91, SF-92, SF-93, SF-94, SF-95, SF-96, SF-97, SF-98, SF-99, SF-100, SF-101, SF-102, SF-103, SF-104, SF-105, SF-106, SF-107, SF-108, SF-109, SF-110, SF-111, SF-112, SF-113, SF-114, SF-115, SF-116, SF-117, SF-118, SF-119, SF-120, SF-121, SF-122, SF-123, SF-124, SF-125, SF-126, SF-127, SF-128, SF-129, SF-130, SF-131, SF-132, SF-133, SF-134, SF-135, SF-136, SF-137, SF-138, SF-139, SF-140, SF-141, SF-142, SF-143, SF-144, SF-145, SF-146, SF-147, SF-148, SF-149, SF-150, SF-151, SF-152, SF-153, SF-154, SF-155, SF-156, SF-157, SF-158, SF-159, SF-160, SF-161, SF-162, SF-163, SF-164, SF-165, SF-166, SF-167, SF-168, SF-169, SF-170, SF-171, SF-172, SF-173, SF-174, SF-175, SF-176, SF-177, SF-178, SF-179, SF-180, SF-181, SF-182, SF-183, SF-184, SF-185, SF-186, SF-187, SF-188, SF-189, SF-190, SF-191, SF-192, SF-193, SF-194, SF-195, SF-196, SF-197, SF-198, SF-199, SF-200, SF-201, SF-202, SF-203, SF-204, SF-205, SF-206, SF-207, SF-208, SF-209, SF-210, SF-211, SF-212, SF-213, SF-214, SF-215, SF-216, SF-217, SF-218, SF-219, SF-220, SF-221, SF-222, SF-223, SF-224, SF-225, SF-226, SF-227, SF-228, SF-229, SF-230, SF-231, SF-232, SF-233, SF-234, SF-235, SF-236, SF-237, SF-238, SF-239, SF-240, SF-241, SF-242, SF-243, SF-244, SF-245, SF-246, SF-247, SF-248, SF-249, SF-250, SF-251, SF-252, SF-253, SF-254, SF-255, SF-256, SF-257, SF-258, SF-259, SF-260, SF-261, SF-262, SF-263, SF-264, SF-265, SF-266, SF-267, SF-268, SF-269, SF-270, SF-271, SF-272, SF-273, SF-274, SF-275, SF-276, SF-277, SF-278, SF-279, SF-280, SF-281, SF-282, SF-283, SF-284, SF-285, SF-286, SF-287, SF-288, SF-289, SF-290, SF-291, SF-292, SF-293, SF-294, SF-295, SF-296, SF-297, SF-298, SF-299, SF-300, SF-301, SF-302, SF-303, SF-304, SF-305, SF-306, SF-307, SF-308, SF-309, SF-310, SF-311, SF-312, SF-313, SF-314, SF-315, SF-316, SF-317, SF-318, SF-319, SF-320, SF-321, SF-322, SF-323, SF-324, SF-325, SF-326, SF-327, SF-328, SF-329, SF-330, SF-331, SF-332, SF-333, SF-334, SF-335, SF-336, SF-337, SF-338, SF-339, SF-340, SF-341, SF-342, SF-343, SF-344, SF-345, SF-346, SF-347, SF-348, SF-349, SF-350, SF-351, SF-352, SF-353, SF-354, SF-355, SF-356, SF-357, SF-358, SF-359, SF-360, SF-361, SF-362, SF-363, SF-364, SF-365, SF-366, SF-367, SF-368, SF-369, SF-370, SF-371, SF-372, SF-373, SF-374, SF-375, SF-376, SF-377, SF-378, SF-379, SF-380, SF-381, SF-382, SF-383, SF-384, SF-385, SF-386, SF-387, SF-388, SF-389, SF-390, SF-391, SF-392, SF-393, SF-394, SF-395, SF-396, SF-397, SF-398, SF-399, SF-400, SF-401, SF-402, SF-403, SF-404, SF-405, SF-406, SF-407, SF-408, SF-409, SF-410, SF-411, SF-412, SF-413, SF-414, SF-415, SF-416, SF-417, SF-418, SF-419, SF-420, SF-421, SF-422, SF-423. A decreased abundance of said one or more SFs in the CSF from the subject relative to CSF

of said one or more SFs in the CSF from the subject relative to CSF from a subject or subjects free from Schizophrenia (e.g., a control sample or a previously determined reference range) indicates the presence of Schizophrenia.

In yet another embodiment, CSF from a subject is analyzed by 2D electrophoresis for quantitative detection of (a) one or more SFs or any combination of them, whose decreased abundance indicates the presence of Schizophrenia, i.e., SF-14, SF-16, SF-17, SF-18, SF-19, SF-20, SF-21, SF-22, SF-23, SF-24, SF-25, SF-26, SF-27, SF-28, SF-29, SF-30, SF-31, SF-32, SF-33, SF-34, SF-35, SF-36, SF-37, SF-38, SF-39, SF-40, SF-41, SF-42, SF-43, SF-44, SF-45, SF-46, SF-47, SF-48, SF-49, SF-50, SF-51, SF-52, SF-53, SF-54, SF-55, SF-56, SF-57, SF-58, SF-59, SF-60, SF-61, SF-62, SF-63, SF-64, SF-65, SF-66, SF-67, SF-68, SF-69, SF-70, SF-71, SF-72, SF-73, SF-74, SF-75, SF-76, SF-77, SF-78, SF-79, SF-80, SF-81, SF-82, SF-83, SF-84, SF-85, SF-86, SF-87, SF-88, SF-89, SF-90, SF-91, SF-92, SF-93, SF-94, SF-95, SF-96, SF-97, SF-98, SF-99, SF-100, SF-101, SF-102, SF-103, SF-104, SF-105, SF-106, SF-107, SF-108, SF-109, SF-110, SF-111, SF-112, SF-113, SF-114, SF-115, SF-116, SF-117, SF-118, SF-119, SF-120, SF-121, SF-122, SF-123, SF-124, SF-125, SF-126, SF-127, SF-128, SF-129, SF-130, SF-131, SF-132, SF-133, SF-134, SF-135, SF-136, SF-137, SF-138, SF-139, SF-140, SF-141, SF-142, SF-143, SF-144, SF-145, SF-146, SF-147, SF-148, SF-149, SF-150, SF-151, SF-152, SF-153, SF-154, SF-155, SF-156, SF-157, SF-158, SF-159, SF-160, SF-161, SF-162, SF-163, SF-164, SF-165, SF-166, SF-167, SF-168, SF-169, SF-170, SF-171, SF-172, SF-173, SF-174, SF-175, SF-176, SF-177, SF-178, SF-179, SF-180, SF-181, SF-182, SF-183, SF-184, SF-185, SF-186, SF-187, SF-188, SF-189, SF-190, SF-191, SF-192, SF-193, SF-194, SF-195, SF-196, SF-197, SF-198, SF-199, SF-200, SF-201, SF-202, SF-203, SF-204, SF-205, SF-206, SF-207, SF-208, SF-209, SF-210, SF-211, SF-212, SF-213, SF-214, SF-215, SF-216, SF-217, SF-218, SF-219, SF-220, SF-221, SF-222, SF-223, SF-224, SF-225, SF-226, SF-227, SF-228, SF-229, SF-230, SF-231, SF-232, SF-233, SF-234, SF-235, SF-236, SF-237, SF-238, SF-239, SF-240, SF-241, SF-242, SF-243, SF-244, SF-245, SF-246, SF-247, SF-248, SF-249, SF-250, SF-251, SF-252, SF-253, SF-254, SF-255, SF-256, SF-257, SF-258, SF-259, SF-260, SF-261, SF-262, SF-263, SF-264, SF-265, SF-266, SF-267, SF-268, SF-269, SF-270, SF-271, SF-272, SF-273, SF-274, SF-275, SF-276, SF-277, SF-278, SF-279, SF-280, SF-281, SF-282, SF-283, SF-284, SF-285, SF-286, SF-287, SF-288, SF-289, SF-290, SF-291, SF-292, SF-293, SF-294, SF-295, SF-296, SF-297, SF-298, SF-299, SF-300, SF-301, SF-302, SF-303, SF-304, SF-305, SF-306, SF-307, SF-308, SF-309, SF-310, SF-311, SF-312, SF-313, SF-314, SF-315, SF-316, SF-317, SF-318, SF-319, SF-320, SF-321, SF-322, SF-323, SF-324, SF-325, SF-326, SF-327, SF-328, SF-329, SF-330, SF-331, SF-332, SF-333, SF-334, SF-335, SF-336, SF-337, SF-338, SF-339, SF-340, SF-341, SF-342, SF-343, SF-344, SF-345, SF-346, SF-347, SF-348, SF-349, SF-350, SF-351, SF-352, SF-353, SF-354, SF-355, SF-356, SF-357, SF-358, SF-359, SF-360, SF-361, SF-362, SF-363, SF-364, SF-365, SF-366, SF-367, SF-368, SF-369, SF-370, SF-371, SF-372, SF-373, SF-374, SF-375, SF-376, SF-377, SF-378, SF-379, SF-380, SF-381, SF-382, SF-383, SF-384, SF-385, SF-386, SF-387, SF-388, SF-389, SF-390, SF-391, SF-392, SF-393, SF-394, SF-395, SF-396, SF-397, SF-398, SF-399, SF-400, SF-401, SF-402, SF-403, SF-404, SF-405, SF-406, SF-407, SF-408, SF-409, SF-410, SF-411, SF-412, SF-413, SF-414, SF-415, SF-416, SF-417, SF-418, SF-419, SF-420, SF-421, SF-422, SF-423. A decreased abundance of said one or more SFs in the CSF from the subject relative to CSF

- 368, SF-369, SF-370, SF-371, SF-372, SF-373, SF-374, SF-375, SF-376, SF-377, SF-378, SF-379, SF-380, SF-381, SF-382, SF-383, SF-384, SF-385, SF-386, SF-387, SF-388, SF-389, SF-390, SF-391, SF-392, SF-393, SF-394, SF-395, SF-396, SF-397, SF-398, SF-399, SF-400, SF-401, SF-402, SF-403, SF-404, SF-405, SF-406, SF-407, SF-408, SF-409, SF-410, SF-411, SF-412, SF-413, SF-414, SF-415, SF-416, SF-417, SF-418, SF-419, SF-420, SF-421, SF-422, SF-423; and (b) one or more SFs or any combination of them, whose increased abundance indicates the presence of Schizophrenia i.e., SF-80, SF-81, SF-82, SF-83, SF-84, SF-85, SF-86, SF-87, SF-88, SF-89, SF-90, SF-91, SF-92, SF-93, SF-94, SF-95, SF-96, SF-97, SF-98, SF-99, SF-100, SF-101, SF-102, SF-103, SF-104, SF-105, SF-106, SF-107, SF-108, SF-109, SF-110, SF-111, SF-112, SF-113, SF-114, SF-115, SF-116, SF-117, SF-118, SF-119, SF-120, SF-121, SF-124, SF-125, SF-126, SF-130, SF-131, SF-132, SF-135, SF-136, SF-137, SF-139, SF-141, SF-142, SF-143, SF-144, SF-147, SF-148, SF-150, SF-151, SF-153, SF-154, SF-155, SF-157, SF-158, SF-159, SF-160, SF-161, SF-162, SF-163, SF-164, SF-165, SF-166, SF-167, SF-168, SF-169, SF-170, SF-171, SF-172, SF-173, SF-174, SF-175, SF-176, SF-177, SF-178, SF-179, SF-180, SF-181, SF-182, SF-183, SF-184, SF-185, SF-186, SF-187, SF-188, SF-189, SF-190, SF-191, SF-194, SF-195, SF-196, SF-197, SF-198, SF-199, SF-200, SF-201, SF-202, SF-203, SF-204, SF-208, SF-209, SF-211, SF-212, SF-213, SF-215, SF-216, SF-217, SF-218, SF-219, SF-220, SF-221, SF-222, SF-226, SF-227, SF-228, SF-229, SF-230, SF-231, SF-232, SF-233, SF-235, SF-237, SF-238, SF-239, SF-242, SF-243, SF-244, SF-248, SF-249, SF-250, SF-255, SF-257, SF-258, SF-261, SF-262, SF-264, SF-265, SF-267, SF-268, SF-269, SF-271, SF-272, SF-273, SF-280, SF-282, SF-283, SF-286, SF-289, SF-291, SF-292, SF-293, SF-294, SF-296, SF-300, SF-301, SF-302, SF-303, SF-304, SF-306, SF-307, SF-309, SF-312, SF-317, SF-320, SF-321, SF-322, SF-324, SF-326, SF-327, SF-332, SF-333, SF-336, SF-340, SF-348, SF-349, SF-352, SF-358, SF-424, SF-425, SF-426, SF-427, SF-428, SF-429, SF-430, SF-431, SF-432, SF-433, SF-434, SF-435, SF-436, SF-437, SF-438, SF-439, SF-440, SF-441, SF-442, SF-443, SF-444, SF-445, SF-446, SF-447, SF-448, SF-449, SF-450, SF-451, SF-452, SF-453, SF-454, SF-455, SF-456, SF-457, SF-458, SF-459, SF-460, SF-461, SF-462, SF-463, SF-464, SF-465, SF-466, SF-467, SF-468, SF-469, SF-470, SF-471, SF-472, SF-473, SF-474, SF-475, SF-476, SF-477, SF-478, SF-479, SF-480, SF-481, SF-482, SF-483, SF-484, SF-485, SF-486, SF-487, SF-488, SF-489, SF-490, SF-491, SF-492, SF-493, SF-494, SF-495, SF-496, SF-497, SF-498, SF-499, SF-500, SF-501, SF-502.

In yet another embodiment of the invention, CSF from a subject is analyzed by 2D electrophoresis for quantitative detection of one or more of the following SPs: SF-14, SF-16, SF-17, SF-18, SF-19, SF-20, SF-21, SF-22, SF-23, SF-24, SF-25, SF-26, SF-27, SF-28, SF-29, SF-30, SF-31, SF-32, SF-33, SF-34, SF-35, SF-36, SF-37, SF-38, SF-39, SF-40, SF-41, SF-42, SF-43, SF-44, SF-45, SF-46, SF-47, SF-48, SF-49, SF-51, SF-52, SF-53, SF-55, SF-56, SF-57, SF-58, SF-80, SF-81, SF-82, SF-83, SF-84, SF-85, SF-86, SF-87, SF-88, SF-89.



- 5 SF-387, SF-388, SF-389, SF-390, SF-391, SF-392, SF-393, SF-394, SF-395, SF-396, SF-397,  
 SF-398, SF-399, SF-400, SF-401, SF-402, SF-403, SF-404, SF-405, SF-406, SF-407, SF-408,  
 SF-409, SF-410, SF-411, SF-412, SF-413, SF-414, SF-415, SF-416, SF-417, SF-418, SF-419,  
 SF-420, SF-421, SF-422, SF-423 are suitable SFs for this purpose. In another specific  
 10 embodiment, an increase in one or more SF/ERF ratios in a test sample relative to the SF/ERF  
 ratios in a control sample or a reference range indicates the presence of Schizophrenia; SF-80,  
 SF-81, SF-82, SF-83, SF-84, SF-85, SF-86, SF-87, SF-88, SF-89, SF-90, SF-91, SF-92, SF-  
 93, SF-94, SF-95, SF-96, SF-97, SF-98, SF-99, SF-100, SF-101, SF-102, SF-103, SF-104,  
 SF-105, SF-106, SF-107, SF-108, SF-109, SF-110, SF-111, SF-112, SF-113, SF-114, SF-115,  
 SF-116, SF-117, SF-118, SF-119, SF-120, SF-123, SF-124, SF-125, SF-126, SF-130, SF-131,  
 SF-132, SF-133, SF-136, SF-137, SF-139, SF-141, SF-142, SF-143, SF-144, SF-147, SF-148,  
 SF-150, SF-151, SF-153, SF-154, SF-155, SF-157, SF-158, SF-159, SF-160, SF-161, SF-162,  
 SF-163, SF-164, SF-165, SF-166, SF-167, SF-168, SF-169, SF-170, SF-171, SF-172, SF-173,  
 SF-174, SF-175, SF-176, SF-177, SF-178, SF-179, SF-180, SF-181, SF-182, SF-183, SF-184,  
 SF-185, SF-186, SF-187, SF-188, SF-189, SF-190, SF-191, SF-194, SF-195, SF-196, SF-197, SF-198,  
 SF-199, SF-200, SF-201, SF-202, SF-203, SF-204, SF-208, SF-209, SF-211, SF-212, SF-213,  
 SF-215, SF-216, SF-217, SF-218, SF-219, SF-220, SF-221, SF-222, SF-226, SF-227, SF-228,  
 SF-229, SF-230, SF-231, SF-232, SF-233, SF-235, SF-237, SF-238, SF-239, SF-242, SF-243,  
 SF-244, SF-248, SF-249, SF-250, SF-255, SF-257, SF-258, SF-261, SF-262, SF-264, SF-265,  
 SF-267, SF-268, SF-269, SF-271, SF-272, SF-273, SF-280, SF-282, SF-283, SF-284, SF-285,  
 SF-291, SF-292, SF-293, SF-294, SF-296, SF-300, SF-301, SF-302, SF-303, SF-304, SF-306,  
 SF-307, SF-309, SF-312, SF-317, SF-320, SF-321, SF-322, SF-324, SF-326, SF-327, SF-332,  
 SF-333, SF-336, SF-340, SF-348, SF-349, SF-352, SF-358, SF-424, SF-425, SF-426, SF-427,  
 SF-428, SF-429, SF-430, SF-431, SF-432, SF-433, SF-434, SF-435, SF-436, SF-437, SF-438,  
 SF-439, SF-440, SF-441, SF-442, SF-443, SF-444, SF-445, SF-446, SF-447, SF-448, SF-449,  
 SF-450, SF-451, SF-452, SF-453, SF-454, SF-455, SF-456, SF-457, SF-458, SF-459, SF-460,  
 SF-461, SF-462, SF-463, SF-464, SF-465, SF-466, SF-467, SF-468, SF-469, SF-470, SF-471,  
 SF-472, SF-473, SF-474, SF-475, SF-476, SF-477, SF-478, SF-479, SF-480, SF-481, SF-482,  
 SF-483, SF-484, SF-485, SF-486, SF-487, SF-488, SF-489, SF-490, SF-491, SF-492, SF-493,  
 SF-494, SF-495, SF-496, SF-497, SF-498, SF-499, SF-500, SF-501, SF-502 are suitable SFs  
 for this purpose.

- 45, SF-46, SF-47, SF-48, SF-49, SF-51, SF-52, SF-53, SF-55, SF-56, SF-57, SF-58, SF-368,  
 SF-369, SF-370, SF-371, SF-372, SF-373, SF-374, SF-375, SF-376, SF-377, SF-378, SF-379,  
 SF-380, SF-381, SF-382, SF-383, SF-384, SF-385, SF-386, SF-387, SF-388, SF-389, SF-390,  
 SF-391, SF-392, SF-393, SF-394, SF-395, SF-396, SF-397, SF-398, SF-399, SF-400, SF-401,  
 SF-402, SF-403, SF-404, SF-405, SF-406, SF-407, SF-408, SF-409, SF-410, SF-411, SF-412,  
 SF-413, SF-414, SF-415, SF-416, SF-417, SF-418, SF-419, SF-420, SF-421, SF-422, SF-423;  
 (b) one or more SFs, or any combination of them, whose increased SF/ERF ratio(s) in a test  
 sample relative to the SF/ERF ratio(s) in a control sample indicates the presence of  
 Schizophrenia, i.e., SF-80, SF-81, SF-82, SF-83, SF-84, SF-85, SF-86, SF-87, SF-88, SF-89,  
 SF-90, SF-91, SF-92, SF-93, SF-94, SF-95, SF-96, SF-97, SF-98, SF-99, SF-100, SF-101,  
 SF-102, SF-103, SF-104, SF-105, SF-106, SF-107, SF-108, SF-109, SF-110, SF-111, SF-112,  
 SF-113, SF-114, SF-115, SF-116, SF-117, SF-118, SF-119, SF-120, SF-123, SF-124, SF-125,  
 SF-126, SF-130, SF-131, SF-132, SF-135, SF-136, SF-137, SF-139, SF-141, SF-142, SF-143,  
 SF-144, SF-147, SF-148, SF-150, SF-151, SF-153, SF-154, SF-155, SF-157, SF-158, SF-159,  
 SF-160, SF-161, SF-162, SF-163, SF-164, SF-165, SF-166, SF-167, SF-168, SF-169, SF-170,  
 SF-171, SF-172, SF-173, SF-174, SF-175, SF-176, SF-177, SF-178, SF-179, SF-180, SF-181,  
 SF-182, SF-183, SF-184, SF-186, SF-187, SF-188, SF-189, SF-190, SF-191, SF-194, SF-195,  
 SF-196, SF-197, SF-198, SF-199, SF-200, SF-201, SF-202, SF-203, SF-204, SF-205, SF-209,  
 SF-211, SF-212, SF-213, SF-215, SF-216, SF-217, SF-218, SF-219, SF-220, SF-221, SF-222,  
 SF-226, SF-227, SF-228, SF-229, SF-230, SF-231, SF-232, SF-233, SF-235, SF-237, SF-238,  
 SF-239, SF-242, SF-243, SF-244, SF-248, SF-249, SF-250, SF-255, SF-257, SF-258, SF-261,  
 SF-262, SF-264, SF-265, SF-267, SF-268, SF-269, SF-271, SF-272, SF-273, SF-280, SF-282,  
 SF-283, SF-286, SF-289, SF-291, SF-292, SF-293, SF-294, SF-296, SF-300, SF-301, SF-302,  
 SF-303, SF-304, SF-306, SF-307, SF-309, SF-312, SF-317, SF-320, SF-321, SF-322, SF-324,  
 SF-326, SF-327, SF-332, SF-333, SF-336, SF-340, SF-348, SF-349, SF-352, SF-358, SF-424,  
 SF-425, SF-426, SF-427, SF-428, SF-429, SF-430, SF-431, SF-432, SF-433, SF-434, SF-435,  
 SF-436, SF-437, SF-438, SF-439, SF-440, SF-441, SF-442, SF-443, SF-444, SF-445, SF-446,  
 SF-447, SF-448, SF-449, SF-450, SF-451, SF-452, SF-453, SF-454, SF-455, SF-456, SF-457,  
 SF-458, SF-459, SF-460, SF-461, SF-462, SF-463, SF-464, SF-465, SF-466, SF-467, SF-468,  
 SF-469, SF-470, SF-471, SF-472, SF-473, SF-474, SF-475, SF-476, SF-477, SF-478, SF-479,  
 SF-480, SF-481, SF-482, SF-483, SF-484, SF-485, SF-486, SF-487, SF-488, SF-489, SF-490,  
 SF-491, SF-492, SF-493, SF-494, SF-495, SF-496, SF-497, SF-498, SF-499, SF-500, SF-501,  
 SF-502.

In a preferred embodiment, CSF from a subject is analyzed for quantitative detection of a plurality of SPs.

## 5.2 Schizophrenia-Associated Protein Isoforms (SPLs)

In another aspect of the invention, CSF from a subject, preferably a living subject, is analyzed for quantitative detection of one or more Schizophrenia-Associated Protein Isoforms (SPIs) for screening or diagnosis of Schizophrenia, to determine the prognosis of a subject having Schizophrenia, to monitor the effectiveness of Schizophrenia therapy, for identifying patients most likely to respond to a particular therapeutic treatment or for drug development. As is well known in the art, a given protein may be expressed as variants (isoforms) that differ in their amino acid composition (e.g. as a result of alternative mRNA or pre-mRNA processing, e.g. alternative splicing or limited proteolysis) or as a result of differential post-translational modification (e.g., glycosylation, phosphorylation, acylation), or both, so that proteins of identical amino acid sequence can differ in their pI, MW, or both. It follows that differential presence of a protein isoform does not require differential expression of the gene encoding the protein in question. As used herein, the term "Schizophrenia-Associated Protein Isoform" refers to a protein isoform that is differentially present in CSF from a subject having Schizophrenia compared with CSF from a subject free from Schizophrenia. As used herein, the term "isoform" also refers to a protein that exists in only a single form, i.e., it is not expressed as several variants.

Two groups of SPIs have been identified by amino acid sequencing of SPIs. SPIs were isolated, subjected to proteolysis, and analyzed by mass spectrometry using the methods and apparatus of the Preferred Technology. One skilled in the art can identify sequence information from protein analyzed by mass spectrometry and/or tandem mass spectrometry using various spectral interpretation methods and database searching tools. Examples of some of these methods and tools can be found at the Swiss Institute of Bioinformatics web site at <http://www.expasy.ch/>, and the European Molecular Biology Laboratory web site at [www.mann.embnet-heidelberg.de/Services/PeptideSearch/](http://www.mann.embnet-heidelberg.de/Services/PeptideSearch/). Identification of SPIs was performed primarily using the SEQUEST search program (Eng et al, *J. Am. Soc. Mass Spectrom.* (1994) 5:976-989) with raw, uninterpreted tandem mass spectra of tryptic digest peptides as described in the Examples, *infra*. The first group consists of SPIs that are decreased in the CSF of subjects having Schizophrenia as compared with the CSF of subjects free from Schizophrenia, where the differential presence is significant. The amino acid sequences of tryptic digest peptides of these SPIs identified by tandem mass spectrometry and database searching as described in the Examples, *infra* are listed in Table IV in addition to the pIs and MWs of these SPIs. For SPI-238 and SPI-140, the partial sequence information for these SPIs derived from tandem mass spectrometry was not found to be described in any known public database. These SPIs are listed as 'NOVEL' in Table IV, and further described below.

Table IV. SPIs Decreased in CSF of Subjects Having Schizophrenia

SF#	SPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides
SF-14	SPI-6	6.24	102603	AASGTQNNVLR, EQTMSECEAGALR
SF-16	SPI-231	4.73	28954	IPTTFENGR
SF-19	SPI-312	8.99	21801	ADGFTEDTVFLPQTDK
SF-20	SPI-352	4.25	64918	DODGEILPR, SAVEMEAEEAAK, QELEDLER
SF-21	SPI-232	7.10	10885	QNLEPLFEGYQYINNLR
SF-22	SPI-7	9.58	20268	TMLLOPAGSLGSYSYR
SF-24	SPI-353	4.81	12637	LVGGPMMDASVEEEGVR
SF-24	SPI-354	4.81	12837	SGFIEEDELGFILK
SF-27	SPI-233	8.14	13763	LVGGPMMDASVEEEGVR, ALDFAVGEGYNK
SF-28	SPI-8	9.25	12001	LVGGPMMDASVEEEGVR, ALDFAVGEGYNK
SF-29	SPI-9	8.89	11749	LVGGPMMDASVEEEGVR, ALDFAVGEGYNK
SF-30	SPI-10	4.52	109372	VESLEQEAAANER, GQLVETHMAR
SF-31	SPI-11	5.43	112518	YLELESSGHRR, TCPTCNDPDFGLVQIK, AFLFQDTPR, NNAHG-YFK, TYFEEGER, LDQCYCER, HNGQINWLENDR, CVTDPQCAQTIR
SF-32	SPI-13	5.43	48238	DTDTGALLFIGK, TVQAVLTVPK, LSYEGEVTK, LAAAVSNFGYDLYR, SSFVAPLEK TSLEDFYLDDEER
SF-32	SPI-234	5.43	48238	VELEDWNGR
SF-33	SPI-355	4.25	106909	VESLEQEAAANER
SF-35	SPI-15	5.03	36795	SWFEEPLVEDMQR, LGPLVEOGR, GEVQAMLGQSTEELR, LEECQAQIR, SELEEQLTPVAEETR
SF-35	SPI-16	5.03	36795	EIDESLOVAER, ASSIIDELFODR, TLLSNLEAK
SF-36	SPI-17	9.58	21021	TMLLOPAGSLGSYSYR
SF-37	SPI-18	6.08	93159	EPGLQWWR, HVVPNEVVQQR
SF-38	SPI-235	5.67	48092	LCTVATLR
SF-38	SPI-236	5.67	48092	SEDTGLDSVATR
SF-38	SPI-19	5.67	48092	DTDTGALLFIGK,

				KTSLEDFYLDDEER, ELLDDTIVTAPOK, LSYEGEVTK, LAAAVSNFGYDLYR, SSFVAPLEK
SF-39	SPI-357	4.67	14570	GLEEELOFSLGSK
SF-40	SPI-20	6.93	27331	KPNLQVFLGK, LSELQDFPLER, GLVSWGNIPCGSK, LYVQGIGCOK, EKFGVYITNVCR, ESSQECSSSVR, YTNWICK
SF-41	SPI-21	5.19	50178	TVDAVLTVPK, LSYEGEVTK, LAAAVSNFGYDLYR, SSFVAPLEK TSLEDFYLDDEER
SF-42	SPI-23	5.98	90092	EPGLQWWR, HVVPNEVVQQR, YETDPANR
SF-43	SPI-26	5.43	49573	TALASGGVLDASGDYR
SF-43	SPI-24	5.43	49573	LAAAVSNFGYDLYR, TSLEDFYLDDEER
SF-43	SPI-25	5.43	49573	LTIGEGQQHHLGGAK, VELEDWNGR, YLOEYNSNNOK, RLDGSVDFK
SF-44	SPI-28	8.16	24182	TMLLOPAGSLGSYSYR, AGQFTEDTIVFLPQTDK, APEAQVSQPNFQDQK
SF-45	SPI-29	5.30	49423	DTDTGALLFIGK , LSYEGEVTK, LAAAVSNFGYDLYR, SSFVAPLEK TSLEDFYLDDEER
SF-45	SPI-30	5.30	49423	EPGEFALLR, TALASGGVLDASGDYR
SF-46	SPI-32	7.38	68161	FYYIYNEK, SGIRVTSPYQIHFTK, LVAYYTLIGASGQR, TNTPGSTVLYR, IPIEDGSGEVLSR
SF-47	SPI-33	4.86	38741	DPDFVPPVR, DCEEGVNSLPGSITK, GYTQCLPR, ROGALELJK, AGDFLEANYMMNLR, KGYTQCLAPR
SF-48	SPI-34	5.11	35613	EIDESLOVAER, ASSIIDELFODR, EISVDCSTNPNPSQAK
SF-48	SPI-35	5.11	35613	SWFEEPLVEDMQR, LGPLVEOGR, OWAGLVEK, GPLVLEQGR,

				GEVGAMLGOSTEELR, LEEDQACIPR, SELEEQLTPVAEETR, AATVGSLAGQPLCIER
SF-49	SPI-36	5.80	23795	TMLLOPAGSLGSYSYR, AGQFTEDTIVFLPQTDK, APEAQVSQPNFQDQK
SF-51	SPI-38	7.10	23117	TMLLOPAGSLGSYSYR, AGQFTEDTIVFLPQTDK, APEAQVSQPNFQDQK
SF-52	SPI-39	6.00	49723	EPGEFALLR, TALASGGVLDASGDYR, YEAAPDPR, VAMHLVCPSR
SF-53	SPI-237	4.72	20882	THPHFVIPYR
SF-55	SPI-238	4.94	59286	NOVEL (cloned)
SF-55	SPI-239	4.94	59286	ALEFLQLHNGR
SF-55	SPI-41	4.94	59286	VLSALAOVQGILLVAQGR, ALQDQLVLAQAK, DPTFIPAPIQAK
SF-56	SPI-240	5.04	57690	NOVEL (cloned)
SF-56	SPI-42	5.04	57690	LPGINAEGR, DDLYVSDAFHK, VAEGTOVLELPFK, EVPLNTIIFMGR
SF-57	SPI-43	5.36	20134	CFLAFIOTK, EQOALQTVCILK, LDTLACEVALLK, TFHEASEDCISR, NWETEITAQPDGGK
SF-58	SPI-241	7.20	19285	APEAQVSQPNFQDQK
SF-58	SPI-44	7.20	19285	LYTLVLTDPDAPSR, CDEPILSNR
SF-368	SPI-401	6.18	105482	GCPTEEGCGER, AASGTQNNVLR
SF-368	SPI-402	6.18	105482	NAVGVSLPR
SF-369	SPI-403	4.39	62654	LPNNVVEESAR
SF-370	SPI-404	7.71	57865	TYTPGSTVLYR, IPIEDGSGEVLSR
SF-372	SPI-405	6.58	14769	LVGGPMMDASVEEEGVR, ALDFAVGEGYNK
SF-373	SPI-406	5.96	99056	VSYNVPLEAR
SF-373	SPI-407	5.96	99056	TGAQELLR
SF-376	SPI-408	5.42	18290	QSLAEASLAETEGR
SF-376	SPI-409	5.42	18290	LEGEACGVYTPR
SF-379	SPI-410	5.12	15174	SELEEQLTPVAEETR, GEVGAMLGOSTEELR
SF-380	SPI-411	8.83	39766	LVGGPMMDASVEEEGVR, ALDFAVGEGYNK

SF-382	SPI-412	8.54	54625	TNTPGSTVLYR, IPIEDGSGEVVLSR
SF-389	SPI-413	5.19	25665	THLAPYSDELR
SF-391	SPI-414	4.53	35202	IPTTFENGR
SF-393	SPI-415	8.79	24182	TMLLOPAGSLGSYSSYR, APECQSVQPNFQDQK, AQGFTEDITVFLPQTDK
SF-396	SPI-416	5.58	23599	SELEEQLTPVAEETR, .
SF-398	SPI-417	5.56	23599	TMLLOPAGSLGSYSSYR, AQGFTEDITVFLPQTDK
SF-397	SPI-418	9.39	11427	LVGGPMMDASVEEEGVR, ALDFAVGEYNK
SF-398	SPI-419	6.32	22090	TMLLOPAGSLGSYSSYR, AQGFTEDITVFLPQTDK
SF-399	SPI-420	8.17	12814	LVGGPMMDASVEEEGVR
SF-402	SPI-421	6.03	13175	GSPAINAVAHFRR
SF-404	SPI-422	4.50	32268	IPTTFENGR
SF-405	SPI-423	5.89	60151	DASGVITFTWTPSSGK, SAVCIGPPEK TFTCTAAYPESK, WLQSGEELPR
SF-406	SPI-424	4.91	38741	KGYTQQLAFR, DICEEOVNLSLPGSITK, AGOFLEANYMNLQR, DFDEVPPVVR,
SF-408	SPI-425	4.91	38741	ASSIIDELFDR, ELDESLOVAER
SF-407	SPI-426	9.35	13879	LVGGPMMDASVEEEGVR
SF-409	SPI-427	4.78	138566	FSSCGGGGSGFAGGGF GSR, NMQDMVEDYR
SF-409	SPI-428	4.78	136568	QYDSILR, EGLDLCVLEDSGR, QFPTPGIR
SF-410	SPI-429	5.13	65925	LCODLGPGAFR, FDPSLTQR
SF-410	SPI-430	5.13	65925	SIEVFGOFGNSK, DGNTLTYVR, DVVLTTTFVDDIK, AEDYNEFSV/R
SF-410	SPI-431	5.13	65925	WLQSGEELPR,
SF-411	SPI-432	9.55	18969	QLYGDGTGVLGR, SLPVSDSVLSLGFQQR
SF-412	SPI-433	4.62	36556	ASSIIDELFQDR
SF-412	SPI-434	4.62	36556	ILEVNNCQIDEER
SF-414*	SPI-435	5.03	65526	LCODLGPGAFR
SF-416	SPI-436	4.99	58394	YTFLERS
SF-416	SPI-437	4.99	58394	EVVAIESDSVQPVPR, MMAVAADTLQR,

SF-418	SPI-438	4.99	58394
SF-418	SPI-438	4.99	58394
SF-416	SPI-439	4.99	58394
SF-417	SPI-440	6.01	21999
SF-420	SPI-441	4.63	27331
SF-421	SPI-442	4.86	153822
SF-422	SPI-443	5.84	65594
SF-423	SPI-444	5.43	143548

The second group comprises SPIs that are increased in the CSF of subjects having Schizophrenia as compared with the CSF of subjects free from Schizophrenia, where the differential presence is significant. The amino acid sequences of tryptic digest peptides of these SPIs identified by tandem mass spectrometry and database searching are listed in Table V in addition to the pIs and MWs of these SPIs. For SPI- 206, the partial sequence information derived from tandem mass spectrometry was not found to be described in any known public database. This SPI is listed as 'NOVIL' in Table V, and further described below.

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Table V. SPIs Increased in CSF of Subjects Having Schizophrenia

SF#	SP#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides
SF-81	SPI-321	5.39	28439	EELVYELNPLDHR, GSFEPVGDAVSK
SF-81	SPI-322	5.39	28439	TMLLOPAGSLGSYSSYR, AQGFTEDITVFLPQTDK
SF-82	SPI-323	9.74	56994	VGDTLNLLNR, TTNIQGINLLFSSR
SF-83	SPI-54	7.65	61870	GGSTSYCTCSETESPRL, QFTTSSTSYYR, ESSHHNPGLAEFFSR
SF-84	SPI-381	6.54	13783	LEEOAQQIR

SF-65	SPI-382	6.60	14652	TMLLOPAGSLGSYSSYR, AQGFTEDITVFLPQTDK
SF-66	SPI-56	7.01	44664	YLDGLTAER
SF-67	SPI-383	5.37	29604	TMLLOPAGSLGSYSSYR, AQGFTEDITVFLPQTDK
SF-67	SPI-384	5.37	29604	TSLEDFYLDDEER
SF-68	SPI-57	6.60	57865	LNGMTIDLOGLR, VGDTLNLLNR
SF-69	SPI-324	6.18	52513	TNTPGSTVLYR, TVMVNNIENPEGIPVK
SF-71	SPI-325	5.64	14171	LEEOAQQIR, LGPLVEOGR, SWFPLVEDMQR
SF-72	SPI-326	7.65	46181	ELTEIDDNNEQISSYK
SF-73	SPI-359	6.61	11467	EFTPPVQAAQYQK, LWVVPWTQR, VNVDAVGGEALGR
SF-73	SPI-360	6.61	11467	QMLNPNQPK
SF-74	SPI-58	4.26	113057	LLDLSLPSDTR, FQPTLTLPR
SF-76	SPI-361	6.05	63583	NFPSPVDAAFR, GECQAEGVLFQGDR
SF-77	SPI-327	7.21	50321	AVLYNRY, SNLDEDIAEENIVSR
SF-78	SPI-362	6.67	45227	FONALLVR, CCAAADDPHECYAK, VPQVSTPTLVEVSR
SF-79	SPI-328	5.65	12549	YGLVITYATPK
SF-100	SPI-242	6.39	14220	LEEOAQQIR, LGPLVEOGR
SF-101	SPI-329	9.28	51958	IPIEDGSGEVLSR
SF-102	SPI-59	5.66	14133	LAAAVSNFGYDLYR
SF-102	SPI-60	5.56	14133	LGPLVEOGR, LEEOAQQIR
SF-107	SPI-243	5.72	26522	AAPSVTLPSSSEELQANK
SF-108	SPI-244	6.12	15430	GLODEDGYR
SF-111	SPI-62	7.92	12182	LVGGPMMDASVEEEGVR, ALDFAVGEYNK
SF-112	SPI-331	5.79	14578	AQGFTEDITVFLPQTDK, TMLLOPAGSLGSYSSYR
SF-114	SPI-332	4.47	13640	LAAAVSNFGYDLYR, ELLDTVTAPQK
SF-115	SPI-63	4.28	39080	TYMLAFDWNDEK, EQLGEFYEAALDCLR, SDVWYTDWK, TEDIYLRL
SF-116	SPI-65	6.85	30358	WLQSGEELPR
SF-118	SPI-334	8.88	50636	FONALLVR
SF-123	SPI-335	5.56	28440	WVNYYFOR

SF-124	SPI-385	6.69	19285	QPPFTDYL
SF-126	SPI-245	6.19	46232	TEAESWYOTK, EYDELNMVK
SF-132	SPI-336	4.82	104557	FARQAEVNR, EEEAEIDLGLQUNASQIR
SF-135	SPI-67	7.23	31838	CSVYFAPSK, GLQDQEDGYR, VEYGFQVK, ITCVLHFTK
SF-143	SPI-337	7.46	43043	FEELTR, SFLVVVNEEDHLR
SF-144	SPI-363	7.26	12594	VLAGFSQDGLAHLNDLK, LLVVPWTQR, GTFLATSELHCOK, EFTPVQAYQK
SF-151	SPI-69	6.28	30170	TSLEDFYLDDEER, SSVAPLEK
SF-153	SPI-338	6.95	14369	LVEVWQLQDDK
SF-153	SPI-339	6.95	14369	EVVADSVWWDVK
SF-154	SPI-365	6.39	12122	EFTPPVQAYQK, WVAGVANALAHK, VHLTPEEK
SF-157	SPI-340	4.65	26063	AQGFTEDITVFLPQTDK
SF-158	SPI-387	4.73	11509	AQGFTEDITVFLPQTDK
SF-158	SPI-388	4.73	11509	VETALEACSLPSSR
SF-159	SPI-73	5.03	16103	LGPLVEOGR, LEEOAQQIR
SF-160	SPI-74	6.35	32268	FACYYPR
SF-161	SPI-75	6.55	12903	QWAGLVEK, LGPLVEOGR, LEEOAQQIR, AATVGSLAGQPLQER
SF-163	SPI-76	5.26	14318	LGPLVEOGR, LEEOAQQIR, AATVGSLAGQPLQER
SF-164	SPI-77	6.98	59466	UNMGITDLOGLR, AEQFQALEK, VGDTLNLLNR
SF-165	SPI-389	6.45	20882	FSNTDOAVGYMLR, LVMGIPFTGR
SF-166	SPI-246	5.78	33716	ELDESLOVAER, EISVDCGSTNNPSQAK
SF-167	SPI-78	5.17	15486	QCTEWQSGQR, VEOAVETEPEPELR, GEVQAMLGOSTEELR, SELEEQLTPVAEETR
SF-168	SPI-390	6.07	31433	SSFVAPLEK, TSLEDFYLDDEER
SF-169	SPI-391	5.76	29267	WVNYYFOR, WVEELMK, SYPELTLK
SF-170	SPI-80	7.50	14319	LGPLVEOGR, LEEOAQQIR,

			AATVGSLAGQPLQER
SF-171	SPI-392	6.69	24664 TMLQPAGSLGSYSYR, AQGFTEDTIVFLPCDK
SF-172	SPI-383	5.68	39422 VVNNYFQR, WVEELMK,
SF-173	SPI-247	6.39	44664 LVAEFDPR
SF-174	SPI-81	6.39	44664 IVGLIQQDTR, SIPQVSPVR
SF-176	SPI-83	6.37	34096 GSPPAINAVAHVFR, TSLEDFYLDEER
SF-176	SPI-248	6.37	34096 ISYEEWAK
SF-176	SPI-249	6.37	34096 MIEYVDR
SF-177	SPI-85	4.46	46679 ALGHLDLSGNR, VAAGAFGGLR, YUFLNGNK
SF-178	SPI-250	7.68	64540 TTYPGSTVLYR
SF-179	SPI-87	6.05	30643 TSLEDFYLDEER
SF-180	SPI-251	6.21	67544 DGFBQDGETMFPVVK
SF-181	SPI-88	6.29	80131 VSVFVPPR
SF-182	SPI-252	4.95	14570 LGADMEDVCGR, GEVOAMLGQSSTEELR, SELEELQTPVAEETR
SF-184	SPI-253	6.52	60192 MLQWDIDICVR
SF-185	SPI-254	7.46	59646 UNMGITDLOGLR, CQIVFMNR, EMSSGPASIPVK
SF-187	SPI-255	7.27	59466 UNMGITDLOGLR, CQIVFMNR, EMSSGPASIPVK, AEFODALEK, VGDTLNLNR
SF-188	SPI-394	7.01	40510 LNDLEEAQOAK
SF-189	SPI-91	6.01	53953 GECQAEGVLFQGDR, DYFMPCPGR
SF-190	SPI-257	4.91	70663 TGYYFDGISR, CLAFECPENYR, IIIEEEEEQEDPYUNDR
SF-190	SPI-258	4.81	70663 FDPSLTQR, LCQDLPGFAFR
SF-191	SPI-92	6.74	64791 QELSEAEOATR, TTYPGSTVLYR, IPIEDGSGEVLSR
SF-191	SPI-259	6.74	64791 ATVYVGER
SF-194	SPI-281	7.03	55966 IPIEDGSGEVLSR
SF-196	SPI-262	5.52	178161 RPYFPVAVVK, SCOPVFMNAR
SF-197	SPI-95	5.32	15381 TMLQPAGSLGSYSYR

SF-197	SPI-93	5.32	15381 LGADMEDVCGR, VEQAVETEPEPELRL, GEVOAMLGQSSTEELR, SELEELQTPVAEETR
SF-198	SPI-96	7.73	15277 EPGLQIWR, HVNPNEVVVR
SF-199	SPI-97	6.28	67135 EQTMSECEAGALR
SF-200	SPI-99	6.03	135312 TLNICEVGTR, QLEWLGLR, HEGSFIGAEK
SF-201	SPI-100	6.10	57515 IAPANADFAFR
SF-202	SPI-101	5.13	42039 YVMLPVADQEK
SF-209	SPI-105	6.53	10226 SCDLALLETYCATPAK, GIVEECCFR
SF-211	SPI-367	6.53	25861 GLVSWGNIPCGSK
SF-212	SPI-263	5.48	179707 TGESVEFCK, IDVHLVPDR
SF-213	SPI-264	4.87	45882 ELDESLOVAER
SF-213	SPI-107	4.87	45882 DHADLQK, TEQWSTLPPETK, VLSLAQEVGGSPEK, OGSSFOGGR, KADGSYAAWLSR, AEMADQAAAWLTR
SF-215	SPI-341	5.55	178161 EIMENYIALR
SF-217	SPI-113	5.03	17230 GEVOAMLGQSSTEELR, KVEQAVETEPEPELRL, SELEELQTPVAEETR
SF-219	SPI-114	6.56	20744 EVDSGNDIYGNPK, SGSGCAWYR
SF-221	SPI-342	6.88	100168 TGAQELLR
SF-222	SPI-115	6.37	66932 AACTONNVLR, ECTMSECEAGALR
SF-222	SPI-265	6.37	66932 YLYEIAR, CCTESLVR
SF-223	SPI-118	5.74	38251 IETALTSHQR, LENLEOYSR
SF-226	SPI-266	4.81	50178 VEQATOQAIMER, CMYPMOLIAR
SF-226	SPI-267	4.81	50178 ATVNPSAPR, VLDSLSCR
SF-227	SPI-268	6.46	52673 VPPTLEVTOCPVVR
SF-227	SPI-269	6.46	52673 IAPANADFAFR, DFYDENTTVR
SF-228	SPI-270	5.97	14520 IWDVVEK, QPVPGQGMTLK
SF-229	SPI-122	7.42	56136 QELSEAEOATR, GLEVITAR, TTYPGSTVLYR, IPIEDGSGEVLSR

SF-230	SPI-123	4.31	63376 DQDGIELLPR, QELEDLER
SF-231	SPI-124	7.81	59828 IPGIFELGISSQSDR, UPLESYGEYR
SF-232	SPI-343	7.31	64759 AVLYNRY
SF-233	SPI-344	5.02	50026 TALASGGVLDASGDYR
SF-233	SPI-345	5.02	50026 WLQGSQELPR
SF-235	SPI-346	4.49	18350 NFFSPVDAFR
SF-238	SPI-127	7.67	104514 WELCDIPR, HSIFTPETNPR, YEFLNGR
SF-242	SPI-129	5.48	11872 FSSCGGGGGSFAGGGF GSR
SF-243	SPI-130	7.65	52513 QDGSVDFGR, LEDSVSAQIMEYCR, EDGGGGWWYNR, QFGFQNVATNTDK
SF-243	SPI-273	7.65	52513 EDDYHYLLDR, GFQQLLQEJNCPR, TLYLADTFTPNR
SF-244	SPI-369	6.65	12463 VHLPKEK, GTFATLSELHCDK, VLGAFSDQLAHDLNK, LLVWPWTQ, EFTPPVQAYQ
SF-248	SPI-370	5.40	11896 LVGGPMASVEEEVGR
SF-249	SPI-274	6.18	178932 TDGEITYQCR
SF-250	SPI-133	5.05	15381 RAKAELAKETDPLRR
SF-250	SPI-132	5.05	15381 VEQAVETEPEPELRL, GEVOAMLGQSSTEELR, LEQAOQCR, SELEELQTPVAEETR
SF-255	SPI-138	7.03	155828 GPPGPGGGVVR, GEIILPCQPR, VELAGDLR, FAQNLAAEDTR
SF-257	SPI-275	5.75	60558 SAVQGPR, WLQGSQELPR, TFTCTAAYPESK, DASGVITFTWTPSSKG
SF-258	SPI-139	5.06	49723 LTVGAAACVPAQDILVGLR
SF-262	SPI-397	6.72	57865 EPGLQIWR, EVOQFESATFLGYFK, HVNPNEVVVR, QTQVSVLPEGETPLFK
SF-264	SPI-141	5.50	151186 VOVTSQEYSAR
SF-265	SPI-142	6.90	156503 GPPGPGGGVVR, FAQNLAAEDTR
SF-267	SPI-143	5.30	43920 SYELPDQGVTIGNER, GYSFTTAER, QYEDESGPSVHR

SF-268	SPI-151	7.22	155156 GPPGPGGGVVR, VISDTEADIGNSLR, VTVPDPGTLLIR, FAQNLAAEDTR, GEIILPCQPR
SF-269	SPI-152	6.18	52038 GECQAEGVLFQGDR, RLWMLDLK, DYFMPCPGR, YYCFQGNQFLR
SF-271	SPI-154	5.06	13452 GSPAINAVAHVFR, AADDTWEFPASCK
SF-272	SPI-155	5.17	64933 FDPSLTQR, LCQDLPGFAFR
SF-273	SPI-348	6.09	67749 VLFDVSEK
SF-280	SPI-164	4.65	45728 TEQWSTLPPETK, VLSLAQEOVGGSPK, OGSSFOGGR, AEMADQAAAWLTR
SF-282	SPI-165	4.86	31780 ESYNVLCLPAPR
SF-283	SPI-167	5.49	60558 SAVQGPR, WLQGSQELPR, DASGVITFTWTPSSKG
SF-286	SPI-169	4.99	61670 YFIDFVAR, YNSONGSNOCFVLYR, TVGSDTFYSFK
SF-286	SPI-170	4.99	61670 QEPSQTTTFAVTSILR, WLQGSQELPR
SF-289	SPI-398	6.28	178161 EIMENYIALR
SF-291	SPI-176	7.14	32649 TSLEDFYLDEER
SF-291	SPI-175	7.14	32549 CSVFGAPSK, GLODEDGR, VEYGFQVK, ITQVLHTK, FACYPR, VHHTWCWR
SF-292	SPI-349	7.27	48975 VVIGMDVAASEFFR
SF-293	SPI-372	9.24	35621 VPTANVSVDLTCR, LISWYDNEFGYSNR
SF-296	SPI-276	6.52	175109 IDVHLVPDR
SF-300	SPI-179	7.39	153822 GPPGPGGGVVR, FAQNLAAEDTR
SF-300	SPI-281	7.39	153822 GPPGPGGGVVR, FAQNLAAEDTR
SF-301	SPI-375	7.14	95262 TTYPGSTVLYR, IPIEDGSGEVLSR
SF-302	SPI-376	5.41	44664 TTYPGSTVLYR, IPIEDGSGEVLSR
SF-303	SPI-181	6.68	40613 ITWSNPPAQGAR, VGVGVSLSGGTGALR, NGFLYNER, HYLLPSGR
SF-304	SPI-182	7.25	67622 IPSETLN, OAGLGNHLSGSR, ILGDPEARL

SF-306	SPI-399	5.72	100168	IEIFOTLPLVR, MILLEAPTSNDNGR
SF-307	SPI-183	8.43	50636	GEOQAEGVLFFQGDR, DVFMPCPGR, YYCFQGNQFLR
SF-309	SPI-185	5.28	72474	GEOQAEGVLFFQGDR, NFPSPVDAFR, WVYVYPPEK
SF-309	SPI-184	5.28	72474	NGVAQEVP/VLDSPAII, ATWSGAVLAGR, CLAPLEGAR, HQFLLTGDTQGR
SF-317	SPI-400	5.59	43773	EGPVJLJGR
SF-320	SPI-189	6.26	21818	TMLLOPAGSLSGSYSR, APEACQVSQVPNFQODK
SF-321	SPI-379	6.72	101681	YGLVITYATVPK
SF-322	SPI-190	6.89	26797	LQNENNIENSCR
SF-324	SPI-193	5.20	43920	ISASAEELR, LAPLAEDVR, ALVOOMEOLR, LEPYADQLR, RVEPYGENFNK
SF-326	SPI-285	4.96	74524	NGVAQEVP/VLDSPAII, HQFLLTGDTQGR, ATWSGAVLAGR
SF-327	SPI-195	4.40	16835	TOSSLVPALTDFV
SF-332	SPI-289	9.05	72071	LNMGTIDLGRLR, SGCHQHQLLR, VGDTLNLLNR
SF-333	SPI-200	4.50	47610	ALGHLDLSGNR, VAAAGFOGQLR
SF-336	SPI-290	7.03	107446	FVTWIEGMVK, YEFLNGR
SF-340	SPI-205	5.03	45659	VLSLAQEQVGGSPEK, QGSQFGGR, AEMADQAAAALTR
SF-342	SPI-206	5.08	29463	NOVEL (cloned)
SF-344	SPI-296	4.76	23795	EVALGLWK, TYGLPCHCPCPK
SF-348	SPI-211	6.30	50790	GEOQAEGVLFFQGDR, WVYVYPPEK, DVFMPCPGR, YYCFQGNQFLR
SF-348	SPI-302	6.30	50790	SYLVAGETATLR
SF-349	SPI-303	7.16	39536	FTWSNPPAOGAR, VGGVOSLGGTGAJR
SF-352	SPI-213	7.09	19543	LYTLVLTDPAPSRS
SF-352	SPI-214	7.09	19543	TMLLOPAGSLSGSYSR, APEACQVSQVPNFQODK
SF-354	SPI-308	4.59	22458	VVEOMCITOYER
SF-424	SPI-445	6.07	177393	TGDEITYQCR

SF-425	SPI-448	8.99	61111	LVGCPMDASVVEEGVR, ALDFAVGEYNK
SF-434	SPI-447	4.41	24762	LPYTAGSLMAPR
SF-440	SPI-448	7.31	64933	GLDENVNDFTRN, ESSSHHPPGAEFFSR,
SF-443	SPI-449	4.24	39855	WFTYASAR, TEDITFLR, YVGCGEHAFLHLR, TYMLAFDVNDEK, NWGLSVYADKPETT, EQLGEFYEAALDCLR, SDVVTDTWK
SF-446	SPI-450	6.14	47484	TALASGGVLDASGDYR, EPEGEFLDR
SF-448	SPI-451	4.34	10961	DQDGIELLPR
SF-451	SPI-452	9.26	17225	LVGCPMDASVVEEGVR
SF-459	SPI-453	8.61	29902	QSLEASLAETEQR
SF-462	SPI-454	5.00	31104	GSPANIVAHVFR, AADDTWEPPFASCK
SF-462	SPI-455	5.00	31104	AEAIGAYPTR
SF-464	SPI-456	8.19	27009	TMLLOPAGSLGSYSYR
SF-471	SPI-457	4.68	15911	LGPLVQDGR, AATVGSLSLAGQPLQER, LEOCACQIR, SWFELPLVEDMQR
SF-472	SPI-458	7.51	24762	EMTOQSPLATLSLSPGER, FSCSGSGCTDFTLTISR, VYACEVTHQGLSSPVTK
SF-472	SPI-459	7.51	24762	TMLLOPAGSLGSYSYR, AQFGTTEDTVPLQPTDK
SF-475	SPI-460	5.68	73979	WBLOQVDTTTR
SF-477	SPI-461	8.04	55531	QELSEAEQPAK
SF-478	SPI-462	6.80	32080	WEEQESR, VHYTCIVWR, CSVFGAPSK, FACYPPR, VEYGFCVVK, TQVLHFTR, GLODEDGYR
SF-487	SPI-463	7.28	34494	TELFPGDR, DNLAQCTR
SF-494	SPI-464	6.69	39193	INHGILYDEEK, EMENNYNIALR
SF-496	SPI-465	6.82	109447	CEDEEFTCR, WELCDIPR
SF-498	SPI-466	6.62	109447	CFELQEAGPPDCR,
SF-502	SPI-467	4.23	39786	WFTYASAR, TEDITFLR, YVGCGEHAFLHLR, TYMLAFDVNDEK, NWGLSVYADKPETT, EQLGEFYEAALDCLR, SDVVTDTWK

As will be evident to one of skill in the art, based upon the present description, a given SPI can be described according to the data provided for that SPI in Table IV or V. The SPI is a protein comprising a peptide sequence described for that SPI (preferably comprising a plurality of, more preferably all of, the peptide sequences described for that SPI) and has a pI of about the value stated for that SPI (preferably within 10%, more preferably within 5% still more preferably within 1% of the stated value) and has a MW of about the value stated for that SPI (preferably within 10%, more preferably within 5%, still more preferably within 1% of the stated value). Proteins comprising the peptide sequences provided in Table IV and V can be identified by searching sequence databases with those peptides using search tools known to those skilled in the art. Examples of search algorithm tools that can be used to identify proteins from peptide sequences include:

- 15 • BLAST (Basic Local Alignment Search Tool) : BLAST is maintained at the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>) and is based on a statistical theory developed by Samuel Karlin and Steven Altschul (*Proc. Natl Acad. Sci. USA* (1990) 87:2284-2268), later modified as in Karlin and Altschul (*Proc. Natl Acad. Sci.* (1993) 90:5873). BLASTP can be used to search a protein sequence against a protein database. TBLASTN can be used to search a Protein Sequence against a Nucleotide Database, by translating each database Nucleotide sequence in all 6 reading frames.

20 • FASTA as described in Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-8. See also Pearson *Methods Enzymol.* (1990) 183:63-98 and Pearson *Genomics* (1991) 11(3):635-50.

Examples of available protein sequence databases include:

The protein database maintained at the National Cen-

- The nr protein database maintained at the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>). The nr protein database is compiled of entries from various sources including SwissProt, SwissProt updates, PIR, and PDB. The BLAST resource is available for sequence searching.
  - SwissProt and TrEMBL database developed by the Swiss Bioinformatics Institute (SIB) and the European can be found at <http://www.expasy.ch>. BLASTP resources are available for sequence searching.
  - The PIR-International Protein Sequence Database maintained by the Protein Information Resource (PIR), in collaboration with the Munich Information Center for Protein Sequences (MIPS) and the Japanese International Protein Sequence Database (JIPID). The Protein Identification Resource (PIR) is a division of the National Biomedical Research Foundation (NBRF) which is affiliated with Georgetown University Medical Center and can be found at <http://www.nbrf.georgetown.edu/pir/searchdb.html>. The database can be searched using BLAST and FASTA search algorithm tools.

- The Protein Data Bank, maintained by Brookhaven National Laboratory (Long Island, New York, USA) which can be found at <http://www.rcsb.org/pdb/>. The FASTA resource is available at this website for sequence searching.

- In one embodiment, CSF from a subject is analyzed for quantitative detection of one or more of the following SPIs: SPI-1, SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-15, SPI-16, SPI-17, SPI-18\*, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-41, SPI-42, SPI-43, SPI-44, SPI-231, SPI-232, SPI-233, SPI-234, SPI-235, SPI-236, SPI-237, SPI-238, SPI-239, SPI-240, SPI-241, SPI-312, SPI-352, SPI-353, SPI-354, SPI-355, SPI-357, SPI-401, SPI-402, SPI-403, SPI-404, SPI-405, SPI-406, SPI-407, SPI-408, SPI-409, SPI-410, SPI-411, SPI-412, SPI-413, SPI-414, SPI-415, SPI-416, SPI-417, SPI-418, SPI-419, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, SPI-427, SPI-428, SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, or any combination of them, wherein a decreased abundance of the SPI or SPIs (or any combination of them) in the CSF from the subject relative to CSF from a subject or subjects free from Schizophrenia (e.g., a control sample or a previously determined reference range) indicates the presence of Schizophrenia.

In another embodiment of the invention, CSF from a subject is analyzed for

- 20 quantitative detection of one or more of the following SPIs: SPI-54, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-95, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-107, SPI-113, SPI-114, SPI-115, SPI-118, SPI-122, SPI-123, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-139, SPI-141, SPI-142, SPI-143, SPI-151, SPI-152, SPI-154, SPI-155, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-189, SPI-190, SPI-193, SPI-195, SPI-200, SPI-205, SPI-206, SPI-211, SPI-213, SPI-214, SPI-242, SPI-243, SPI-244, SPI-245, SPI-246, SPI-247, SPI-248, SPI-249, SPI-250, SPI-251, SPI-252, SPI-253, SPI-254, SPI-255, SPI-257, SPI-258, SPI-259, SPI-261, SPI-262, SPI-263, SPI-264, SPI-265, SPI-266, SPI-267, SPI-268, SPI-269, SPI-270, SPI-273, SPI-274, SPI-275, SPI-276, SPI-281, SPI-285, SPI-289, SPI-290, SPI-296, SPI-302, SPI-303, SPI-306, SPI-321, SPI-322, SPI-323, SPI-324, SPI-325, SPI-326, SPI-327, SPI-328, SPI-329, SPI-331, SPI-332, SPI-334, SPI-335, SPI-336, SPI-337, SPI-338, SPI-339, SPI-340, SPI-341, SPI-342, SPI-343, SPI-344, SPI-345, SPI-346, SPI-348, SPI-349, SPI-339, SPI-360, SPI-361, SPI-362, SPI-363, SPI-365, SPI-367, SPI-369, SPI-370, SPI-372, SPI-373, SPI-376, SPI-379, SPI-381, SPI-382, SPI-383, SPI-384, SPI-385, SPI-387, SPI-388, SPI-389, SPI-390, SPI-391, SPI-392, SPI-393, SPI-394, SPI-397, SPI-398, SPI-399, SPI-400, SPI-445, SPI-446

SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, SPI-467; or any combination of them, wherein an increased abundance of the SPI or SPIs (or any combination of them) in CSF from the subject relative to CSF from a subject or subjects free from Schizophrenia (e.g., a control sample or a previously determined reference range) indicates the presence of Schizophrenia.

In a further embodiment, CSF from a subject is analyzed for quantitative detection of (a) one or more SPIs, or any combination of them, whose decreased abundance indicates the presence of Schizophrenia, i.e., SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-15, SPI-16, SPI-17, SPI-18, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-41, SPI-42, SPI-43, SPI-44, SPI-45, SPI-46, SPI-47, SPI-48, SPI-49, SPI-50, SPI-51, SPI-52, SPI-53, SPI-54, SPI-55, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-95, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-107, SPI-113, SPI-114, SPI-115, SPI-118, SPI-122, SPI-123, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-139, SPI-141, SPI-142, SPI-143, SPI-151, SPI-152, SPI-154, SPI-155, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-189, SPI-190, SPI-191, SPI-195, SPI-200, SPI-205, SPI-206, SPI-211, SPI-213, SPI-214, SPI-242, SPI-243, SPI-244, SPI-245, SPI-246, SPI-247, SPI-248, SPI-249, SPI-250, SPI-251, SPI-252, SPI-253, SPI-254, SPI-255, SPI-257, SPI-258, SPI-259, SPI-261, SPI-262, SPI-263, SPI-264, SPI-265, SPI-266, SPI-267, SPI-268, SPI-269, SPI-270, SPI-273, SPI-274, SPI-275, SPI-278, SPI-281, SPI-285, SPI-289, SPI-290, SPI-296, SPI-302, SPI-303, SPI-306, SPI-321, SPI-322, SPI-323, SPI-324, SPI-325, SPI-326, SPI-327, SPI-328, SPI-329, SPI-331, SPI-332, SPI-334, SPI-335, SPI-336, SPI-337, SPI-338, SPI-339, SPI-340, SPI-341, SPI-342, SPI-343, SPI-344, SPI-345, SPI-346, SPI-348, SPI-349, SPI-359, SPI-360, SPI-361, SPI-362, SPI-363, SPI-365, SPI-367, SPI-369, SPI-370, SPI-372, SPI-375, SPI-376, SPI-379, SPI-381, SPI-382, SPI-383, SPI-384, SPI-385, SPI-387, SPI-388, SPI-389, SPI-390, SPI-391, SPI-392, SPI-393, SPI-394, SPI-397, SPI-398, SPI-399; SPI-400, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455,

SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, SPI-467.

In yet a further embodiment, CSF from a subject is analyzed for quantitative detection of one or more SPIs and one or more previously known biomarkers of Schizophrenia (e.g., candidate markers such as hypersensitive platelet glutamate receptors (Berk et al, *Int Clin Psychopharmacol* (1999) 14:199-122)). In accordance with this embodiment, the abundance of each SPI and known biomarker relative to a control or reference range indicates whether a subject has Schizophrenia.

Preferably, the abundance of an SPI is normalized to an Expression Reference Protein Isoform (ERPI). ERPIs can be identified by partial amino acid sequencing of ERPs, which are described above, using the methods and apparatus of the Preferred Technology. The partial amino acid sequences of an ERPI, and the known proteins to which it is homologous is presented in Table VI.

15 Table VI. Expression Reference Protein Isoforms

ERP#	ERPI #	Amino Acid Sequences of Tryptic Digest Peptides
ERP-2	ERPI-1	TGAGELRK
ERP-2	ERPI-2	TMLLOPAGSLGSYSYR, AQGFTEDITVFLPCQTDK

As shown above, the SPIs described herein include previously unknown proteins, as well as isoforms of known proteins where the isoforms were not previously known to be associated with Schizophrenia. For each SPI, the present invention additionally provides: (a) a preparation comprising the isolated SPI; (b) a preparation comprising one or more fragments of the SPI; and (c) antibodies that bind to said SPI, to said fragments, or both to said SPI and to said fragments. As used herein, an SPI is "isolated" when it is present in a preparation that is substantially free of contaminating proteins, i.e., a preparation in which less than 10% (preferably less than 5%, more preferably less than 1%) of the total protein present is contaminating protein(s). A contaminating protein is a protein or protein isoform having a significantly different pI or MW from those of the isolated SPI, as determined by 2D electrophoresis. As used herein, a "significantly different" pI or MW is one that permits the contaminating protein to be resolved from the SPI on 2D electrophoresis, performed according to the Reference Protocol.

In one embodiment, an isolated protein is provided, said protein comprising a peptide with the amino acid sequence identified in Table IV or V for an SPI, said protein having a pI

and MW within 10% (preferably within 5%, more preferably within 1%) of the values identified in Table IV or V for that SPI.

The SPIs of the invention can be qualitatively or quantitatively detected by any method known to those skilled in the art, including but not limited to the Preferred Technology described herein, kinase assays, enzyme assays, binding assays and other functional assays, immunoassays, and western blotting. In one embodiment, the SPIs are separated on a 2-D gel by virtue of their MWs and pIs and visualized by staining the gel. In one embodiment, the SPIs are stained with a fluorescent dye and imaged with a fluorescence scanner. Sypro Red (Molecular Probes, Inc., Eugene, Oregon) is a suitable dye for this purpose. A preferred fluorescent dye is Pyridinium, 4-[2-[4-(dipentylamino)-2-trifluoromethylphenyl] ethenyl]-1-(sulfonylbutyl)-, inner salt. See U.S. Application No. 09/412,168, filed on October 5, 1999, which is incorporated herein by reference in its entirety.

Alternatively, SPIs can be detected in an immunoassay. In one embodiment, an immunoassay is performed by contacting a sample from a subject to be tested with an anti-SPI antibody under conditions such that immunospecific binding can occur if the SPI is present, and detecting or measuring the amount of any immunospecific binding by the antibody. Anti-SPI antibodies can be produced by the methods and techniques taught herein; examples of such antibodies known in the art are set forth in Table VII. These antibodies shown in Table VII are already known to bind to the protein of which the SPI is itself a family member. Preferably, the anti-SPI antibody preferentially binds to the SPI rather than to other isoforms of the same protein. In a preferred embodiment, the anti-SPI antibody binds to the SPI with at least 2-fold greater affinity, more preferably at least 5-fold greater affinity, still more preferably at least 10-fold greater affinity, than to said other isoforms of the same protein.

SPIs can be transferred from the gel to a suitable membrane (e.g., a PVDF membrane) and subsequently probed in suitable assays that include, without limitation, competitive and non-competitive assay systems using techniques such as western blots and "sandwich" immunoassays using anti-SPI antibodies as described herein, e.g., the antibodies identified in Table VII, or others raised against the SPIs of interest. The immunoblots can be used to identify those anti-SPI antibodies displaying the selectivity required to immuno-specifically differentiate an SPI from other isoforms encoded by the same gene.

Table VII. Known Antibodies That Recognize SPIs or SPI-Related Polypeptides

SPI#	Antibody	Manufacturer	Catalogue No.
SPI-6	CT Complement, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- G34

SPI-8	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
SPI-9	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
SPI-10	Anti-Alzheimer precursor protein A4	RDI RESEARCH DIAGNOSTICS, INC	RDI-ALZHPA4abm
SPI-15	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YMG- 5029
SPI-16	Goat anti-Clusterrin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
SPI-18	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
SPI-23	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
SPI-32	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-33	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-34	Goat anti-Clusterrin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
SPI-35	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YMG- 5029
SPI-41	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	SC-578
SPI-42	Antithrombin III, Clone: BL-ATII/3, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BYA- 9009-1
SPI-43	Tetramedin, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 494
SPI-54	Monoclonal anti-human Fibrinogen	BIODESIGN INTERNATIONAL	N77190M
SPI-57	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-60	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YMG- 5029
SPI-62	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
SPI-63	Alpha-1-Acid Glycoprotein, Clone: AGP-47, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BYA- 8189-1
SPI-67	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-73	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YMG- 5029
SPI-74	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-033-02

SPI-75	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
SPI-76	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
SPI-77	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-78	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
SPI-80	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
SPI-82	Transferrin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 183
SPI-91	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
SPI-92	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-93	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
SPI-96	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
SPI-97	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- G34
SPI-99	C3 Complement, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- G33
SPI-100	Monoclonal anti-Prokalikrein Heavy Chain	BIODESIGN INTERNATIONAL	N55199M
SPI-101	GorLant-Haptoglobin	BIODESIGN INTERNATIONAL	L15320G
SPI-105	Insulin Like Growth Factor II (IGF-II), Clone: W2H1, Mab anti-, frozen, IMA/ELISA/RIA	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MAS- 970p
SPI-107	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-113	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
SPI-114	Transferrin, Beta-1, Rabbit anti-Human, IgM, Clone: 3D12, Mab anti-Human, IgM, (NO X w/TMP1), Clone: 3-4, Mab anti-Human, paraffin, IgH	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA498
SPI-115	C7 Complement, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- G34
SPI-122	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-124	C3 Complement, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- G35

SPI-127	Monoclonal mouse anti-human plasminogen	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK4P11-4D2
SPI-129	Monoclonal Rabbit anti-Human Cytokeratin 1 (Keratin 1)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CYTOK1abz
SPI-130	Fibrinogen, Fibrin I, B-beta chain (B3-I-42), Clone: 18C8, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	NYB- 18C8
SPI-132	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
SPI-143	Actin, beta, Clone AC-74, Mab anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BYA- 6553-1
SPI-152	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
SPI-154	Transferrin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 183
SPI-155	Sheep anti-Alpha 2 Antiplasmin	BIODESIGN INTERNATIONAL	K60038C
SPI-164	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-167	Monoclonal mouse anti-Human IgA1	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK1A2-2B5
SPI-170	Monoclonal mouse anti-human IgA1	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK1A2-2B5
SPI-175	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-183	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
SPI-184	Alpha-1-Acid Glycoprotein, Clone: AGP-47, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BYA- 6183-1
SPI-185	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
SPI-190	Factor H (Complement), Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-068-02
SPI-193	Apolipoprotein A (HDL), Sheep anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	ACL- 20075AP
SPI-205	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-211	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
SPI-231	Apolipoprotein D, Clone: 38C8, Mab anti-Human, paraffin, IHWB	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA457
SPI-233	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
SPI-237	Anti-Alzheimer precursor protein A4	RDI RESEARCH DIAGNOSTICS, INC	RDI-ALZHPA4abm
SPI-242	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029

SPI-244	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-245	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
SPI-252	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
SPI-254	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-255	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-258	Sheep anti-Alpha 2 Antiplasmin	BIODESIGN INTERNATIONAL	K60038C
SPI-261	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-264	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
SPI-265	Albumin, Human, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-028-02
SPI-269	Monoclonal anti-Prokalikrein Heavy Chain	BIODESIGN INTERNATIONAL	N55199M
SPI-275	Monoclonal mouse anti-human, IgA1	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK1A2-2B5
SPI-285	Alpha-1-Acid Glycoprotein, Clone: AGP-47, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BYA- 6183-1
SPI-289	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-290	Monoclonal mouse anti-human plasminogen	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK4P11-4D2
SPI-321	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-323	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-325	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
SPI-328	ANTI-CYTOKERATIN TYPE 10	RDI RESEARCH DIAGNOSTICS, INC	RDI-CBL196
SPI-327	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-328	Complement Factor B, C3 proactivator, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 4862
SPI-329	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-334	Albumin, Human, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-028-02
SPI-339	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-342	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210

SPI-343	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-345	Monoclonal mouse anti-human IgA1	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK1A2-2B5
SPI-346	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
SPI-347	ANTI-CYTOKERATIN TYPE 10	RDI RESEARCH DIAGNOSTICS, INC	RDI-CBL196
SPI-348	C7 Complement, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- G34
SPI-349	Monoclonal anti-Neuron Specific Enolase	BIODESIGN INTERNATIONAL	M37403M
SPI-353	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
SPI-355	Anti-Alzheimer precursor protein A4	RDI RESEARCH DIAGNOSTICS, INC	RDI-ALZHPA4abm
SPI-357	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
SPI-361	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
SPI-352	Albumin, Human, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-028-02
SPI-370	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
SPI-372	Glyceraldehyde-3-Phosphate Dehydrogenase	BIODESIGN INTERNATIONAL	H86504M
SPI-375	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-376	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-379	Complement Factor B, C3 proactivator, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 4862
SPI-381	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
SPI-397	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
SPI-402	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
SPI-404	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
SPI-405	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
SPI-407	Gelatin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
SPI-408	ANTI-CYTOKERATIN TYPE 10	RDI RESEARCH DIAGNOSTICS, INC	RDI-CBL196
SPI-409	RABBIT anti-human INSULIN GROWTH FACTOR BINDING PROTEIN 2	RDI RESEARCH DIAGNOSTICS, INC	RDI-IGFBP2abm

	PROTEIN 2	
SPI-410	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION YM- 5029
SPI-411	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION AXL- 574
SPI-412	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION IAMS- 01-001-02
SPI-413	Apolipoprotein A (HDL), Sheep anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION ACL- 20075AP
SPI-414	Apolipoprotein D, Clone: 36C6, Mab anti-Human, paraffin, IH/WB	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION MED- CLA457
SPI-415	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION YM- 5029
SPI-416	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION AXL- 574
SPI-420	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION AXL- 574
SPI-421	Transferrin, Prealbumin, B5K, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION MED- CLA 193
SPI-422	Apolipoprotein D, Clone: 36C6, Mab anti-Human, paraffin, IH/WB	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION MED- CLA457
SPI-423	Monoclonal mouse anti-human IgA1	RDI RESEARCH DIAGNOSTICS, INC RDI-TRK1A2-285
SPI-424	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION IAMS- 01-001-02
SPI-425	Goat anti-Clustein (human)	RDI RESEARCH DIAGNOSTICS, INC RDI-CLUSTRCabG
SPI-426	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION AXL- 574
SPI-427	Polyclonal Rabbit anti-Human Cytokeratin 1 (Keratin 1)	RDI RESEARCH DIAGNOSTICS, INC RDI-CYTOK1abr
SPI-429	Sheep anti-Alpha 2 Antiplasmin	BIODESIGN INTERNATIONAL K90038C
SPI-431	Monoclonal mouse anti-human IgA1	RDI RESEARCH DIAGNOSTICS, INC RDI-TRK1A2-285
SPI-432	C8 Complement, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION
SPI-433	Goat anti-Clustein (human)	RDI RESEARCH DIAGNOSTICS, INC RDI-CLUSTRCabG
SPI-435	Sheep anti-Alpha 2 Antiplasmin	BIODESIGN INTERNATIONAL K90038C
SPI-438	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES sc-579
SPI-441	Apolipoprotein D, Clone: 36C6, Mab anti-Human, paraffin, IH/WB	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION MED- CLA457
SPI-443	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES sc-579

SPI-446	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION AXL- 574
SPI-448	Monoclonal anti-human Fibronogen	BIODESIGN INTERNATIONAL N77190M
SPI-449	Alpha-1-Acid Glycoprotein, Clone: AGP-47, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION BYA- 6189-1
SPI-452	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION AXL- 574
SPI-453	ANTI-CYTOKERATIN TYPE 10	RDI RESEARCH DIAGNOSTICS, INC RDI-CBL198
SPI-454	Transferrin, Prealbumin, B5K, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION MED- CLA 193
SPI-457	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION YM- 5029
SPI-461	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION IAMS- 01-001-02
SPI-462	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION IAMS- 01-032-02
SPI-464	Factor H (Complement), Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION IAMS- 01-056-02
SPI-465	Monoclonal mouse anti-human Plasminogen	RDI RESEARCH DIAGNOSTICS, INC RDI-TRK4P11-4D2
SPI-467	Alpha-1-Acid Glycoprotein, Clone: AGP-47, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION BYA- 6189-1

In one embodiment, binding of antibody in tissue sections can be used to detect aberrant SPI localization or an aberrant level of one or more SPIs. In a specific embodiment, antibody to an SPI can be used to assay a tissue sample (e.g., a brain biopsy) from a subject for the level of the SPI where an aberrant level of SPI is indicative of Schizophrenia. As used herein, an "aberrant level" means a level that is increased or decreased compared with the level in a subject free from Schizophrenia or a reference level. If desired, the comparison can be performed with a matched sample from the same subject, taken from a portion of the body not affected by Schizophrenia.

Any suitable immunoassay can be used, including, without limitation, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement fixation assays, immunoradiometric assays, fluorescent immunoassays and protein A immunoassays.

For example, an SPI can be detected in a fluid sample (e.g., CSF, blood, urine, or tissue homogenate) by means of a two-step sandwich assay. In the first step, a capture

reagent (e.g., an anti-SPI antibody) is used to capture the SPI. Examples of such antibodies known in the art are set forth in Table VII. The capture reagent can optionally be immobilized on a solid phase. In the second step, a directly or indirectly labelled detection reagent is used to detect the captured SPI. In one embodiment, the detection reagent is a lectin. Any lectin can be used for this purpose that preferentially binds to the SPI rather than to other isoforms that have the same core protein as the SPI or to other proteins that share the antigenic determinant recognized by the antibody. In a preferred embodiment, the chosen lectin binds to the SPI with at least 2-fold greater affinity, more preferably at least 5-fold greater affinity, still more preferably at least 10-fold greater affinity, than to said other isoforms that have the same core protein as the SPI or to said other proteins that share the antigenic determinant recognized by the antibody. Based on the present description, a lectin that is suitable for detecting a given SPI can readily be identified by methods well known in the art, for instance upon testing one or more lectins enumerated in Table I on pages 158-159 of Sumar et al, *Lectins as Indicators of Disease-Associated Glycoforms*, In: Gabius H-J & Gabius S (eds), 1993, Lectins and Glycobiology, at pp. 158-174 (which is incorporated herein by reference in its entirety). Lectins with the desired oligosaccharide specificity can be identified, for example, by their ability to detect the SPI in a 2D gel, in a replica of a 2D gel following transfer to a suitable solid substrate such as a nitrocellulose membrane, or in a two-step assay following capture by an antibody. In an alternative embodiment, the detection reagent is an antibody, e.g., an antibody that immunospecifically detects other post-translational modifications, such as an antibody that immunospecifically binds to phosphorylated amino acids. Examples of such antibodies include those that bind to phosphotyrosine (BD Transduction Laboratories, catalog nos.: P11230-050/P11230-150; P11120; P38820; P39020), those that bind to phosphoserine (Zymed Laboratories Inc., South San Francisco, CA, catalog no. 61- 8100) and those that bind to phosphothreonine (Zymed Laboratories Inc., South San Francisco, CA, catalog nos. 71-8200, 13-9200).

If desired, a gene encoding an SPI, a related gene, or related nucleic acid sequences or subsequences, including complementary sequences, can also be used in hybridization assays. A nucleotide encoding SPI, or subsequence thereof comprising at least 8 nucleotides, preferably at least 12 nucleotides, and most preferably at least 15 nucleotides can be used as a hybridization probe. Hybridization assays can be used for detection, prognosis, diagnosis, or monitoring of conditions, disorders, disease states, associated with aberrant expression of genes encoding SPIs, or for differential diagnosis of subjects with signs or symptoms suggestive of Schizophrenia. In particular, such a hybridization assay can be carried out by a method comprising contacting a subject's sample containing nucleic acid with a nucleic acid probe capable of hybridizing to a DNA or RNA that encodes an SPI, under conditions such

that hybridization can occur, and detecting or measuring any resulting hybridization. Nucleotides can be used for therapy of subjects having Schizophrenia, as described below.

The methods and compositions for clinical screening, diagnosis and prognosis of Schizophrenia in a mammalian subject may be diagnostic of Schizophrenia or indicative of Schizophrenia.

Diagnostic methods and compositions are based on Schizophrenia-Associated Features (SFs) and Schizophrenia-Associated Protein Isoforms (SPIs) which are specifically and particularly associated with Schizophrenia and are generally not associated with other diseases or conditions. Such diagnostic SFs or SPIs, which are specifically associated with Schizophrenia, are useful in screening, diagnosis and prognosis as indicators of Schizophrenia. The administration of therapeutic compositions which are directed against or lead to modulation of diagnostic markers may have therapeutic value particularly in Schizophrenia.

Indicative methods and compositions are based on Schizophrenia-Associated Features (SFs) and Schizophrenia-Associated Protein Isoforms (SPIs) which are associated with Schizophrenia but may not be specific only for Schizophrenia, and may be associated with one or more other diseases or conditions. Such indicative SFs or SPIs, which are associated with Schizophrenia, but not only with Schizophrenia, are useful in screening, diagnosis and prognosis as indicators of Schizophrenia. Indicative methods and compositions are particularly useful in the initial or general screening, diagnosis and prognosis of an individual subject, whereby a first indication of a subset of conditions or diseases, including Schizophrenia, is thereby provided. Additional assessment utilizing diagnostic or particular Schizophrenia SFs or SPIs may then be undertaken to provide specific, diagnostic screening, diagnosis and prognosis of the individual subject. The administration of therapeutic

compositions which are directed against or lead to modulation of indicative markers may have therapeutic value in Schizophrenia and other disorders as well, or may be useful therapeutically in more than one disease or condition.

Thus, a diagnostic marker changes (increases, decreases or otherwise alters form or character) significantly in only a single disease or condition or in only a small number of conditions, particularly in related conditions. One such diagnostic marker, SF-329, is provided below in Table VIII.

Table VIII. Example of a diagnostic marker for Schizophrenia:

Feature #	Isoform #	Fold Change	pI	MW (Da)
SF-329	SPI-287	2.1	5.48	85680

An indicative marker changes (increases, decreases or otherwise alters form or character) significantly in more than one condition, particularly in Schizophrenia and one or more other distinct diseases or conditions. One such indicative marker, SF-255, is found to increase in Schizophrenia and is provided in Table IX. This same marker, identified or characterized by the same pI and MW, is noted as DF-155 and similarly found to be increased in Bipolar Affective Disorder (BAD) and Unipolar Depression. The SF-255/DF-155 marker is therefore indicative of Schizophrenia and/or Depression.

Table IX: Example of an indicative marker for Schizophrenia:

Feature #	Isoform #	Disease	Fold Change	pI	MW (Da)
SF-255	SP1-138	Schizophrenia	2.25	7.03	155828
DF-155	DPI-93	Depression	1.92	7.03	155828

The invention also provides diagnostic kits, comprising an anti-SPI antibody. In addition, such a kit may optionally comprise one or more of the following: (1) instructions for using the anti-SPI antibody for diagnosis, prognosis, therapeutic monitoring or any combination of these applications; (2) a labelled binding partner to the antibody; (3) a solid phase (such as a reagent strip) upon which the anti-SPI antibody is immobilized; and (4) a label or insert indicating regulatory approval for diagnostic, prognostic or therapeutic use or any combination thereof. If no labelled binding partner to the antibody is provided, the anti-SPI antibody itself can be labelled with a detectable marker, e.g., a chemiluminescent, enzymatic, fluorescent, or radioactive moiety.

The invention also provides a kit comprising a nucleic acid probe capable of hybridizing to RNA encoding an SPI. In a specific embodiment, a kit comprised in one or more containers a pair of primers (e.g., each in the size range of 6-30 nucleotides, more preferably 10-30 nucleotides and still more preferably 10-20 nucleotides) that under appropriate reaction conditions can prime amplification of at least a portion of a nucleic acid encoding an SPI, such as by polymerase chain reaction (see, e.g., Innis et al., 1990, PCR Protocols, Academic Press, Inc., San Diego, CA), ligase chain reaction (see EP 320,308) use of Qβ replicase, cyclic probe reaction, or other methods known in the art.

Kits are also provided which allow for the detection of a plurality of SFs or a plurality of nucleic acids each encoding an SPI. A kit can optionally further comprise a predetermined amount of an isolated SPI protein or a nucleic acid encoding an SPI, e.g., for use as a standard or control.

or SPI is detectable by the detection method of choice. For example, if an SF is detectable in 95 percent of samples from diseased subjects, the percentage feature presence of that SF in that sample group is 95 percent. If only 5 percent of samples from non-diseased subjects have detectable levels of the same SF, detection of that SF in the sample of a subject would suggest that it is likely that the subject suffers from Schizophrenia.

#### 5.4 Use in Clinical Studies

The diagnostic methods and compositions of the present invention can assist in monitoring a clinical study, e.g., to evaluate drugs for therapy of Schizophrenia. In one embodiment, candidate molecules are tested for their ability to restore SF or SPI levels in a subject having Schizophrenia to levels found in subjects free from Schizophrenia or, in a treated subject (e.g., after treatment with Haloperidol, Pimozide, Perazine, Risperidol, Famotidine, Zyprexa, Clozapine, Mesoridazine, Quetiapine, atypical anti-psychotic medications of Risperidone, Olanzapine and Clozapine and any other Dibenzothiazepines), to preserve SF or SPI levels at or near non-Schizophrenia values. The levels of one or more SFs or SPIs can be assayed.

In another embodiment, the methods and compositions of the present invention are used to screen candidates for a clinical study to identify individuals having Schizophrenia; such individuals can then be either excluded from or included in the study or can be placed in a separate cohort for treatment or analysis. If desired, the candidates can concurrently be screened to identify individuals with Schizophrenia; procedures for these screens are well known in the art.

#### 5.5 Purification of SPIs

In particular aspects, the invention provides isolated mammalian SPIs, preferably human SPIs, and fragments thereof which comprise an antigenic determinant (i.e., can be recognized by an antibody) or which are otherwise functionally active, as well as nucleic acid sequences encoding the foregoing. "Functionally active" as used herein refers to material displaying one or more functional activities associated with a full-length (wild-type) SPI, e.g., binding to an SPI substrate or SPI binding partner, antigenicity (binding to an anti-SPI antibody), immunogenicity, enzymatic activity and the like.

In specific embodiments, the invention provides fragments of an SPI comprising at least 5 amino acids, at least 10 amino acids, at least 50 amino acids, or at least 75 amino acids. Fragments lacking some or all of the regions of an SPI are also provided, as are proteins (e.g., fusion proteins) comprising such fragments. Nucleic acids encoding the foregoing are provided.

#### 5.3 Statistical Techniques for Identifying SFs and SPI Clusters

The uni-variate differential analysis tools, such as fold changes, wilcoxon rank sum test and t-test, are useful in identifying individual SFs or SPIs that are diagnostically associated with Schizophrenia or in identifying individual SPIs that regulate the disease process. In most cases, however, those skilled in the art appreciate that the disease process is associated with a combination of SFs or SPIs (and to be regulated by a combination of SPIs), rather than individual SFs and SPIs in isolation. The strategies for discovering such combinations of SFs and SPIs differ from those for discovering individual SFs and SPIs. In such cases, each individual SF and SPI can be regarded as one variable and the disease can be regarded as a joint, multi-variate effect caused by interaction of these variables.

The following steps can be used to identify markers from data produced by the Preferred Technology.

The first step is to identify a collection of SFs or SPIs that individually show significant association with Schizophrenia. The association between the identified SFs or SPIs and Schizophrenia need not be as highly significant as is desirable when an individual SF or SPI is used as a diagnostic. Any of the tests discussed above (fold changes, wilcoxon rank sum test, etc.) can be used at this stage. Once a suitable collection of SFs or SPIs has been identified, a sophisticated multi-variate analysis capable of identifying clusters can then be used to estimate the significant multivariate associations with Schizophrenia.

Linear Discriminant Analysis (LDA) is one such procedure, which can be used to detect significant association between a cluster of variables (i.e., SFs or SPIs) and Schizophrenia. In performing LDA, a set of weights is associated with each variable (i.e., SF or SPI) so that the linear combination of weights and the measured values of the variables can identify the disease state by discriminating between subjects having Schizophrenia and subjects free from Schizophrenia. Enhancements to the LDA allow stepwise inclusion (or removal) of variables to optimize the discriminant power of the model. The result of the LDA is therefore a cluster of SFs or SPIs which can be used, without limitation, for diagnosis, prognosis, therapy or drug development. Other enhanced variations of LDA, such as Flexible Discriminant Analysis permit the use of non-linear combinations of variables to discriminate a disease state from a normal state. The results of the discriminant analysis can be verified by post-hoc tests and also by repeating the analysis using alternative techniques such as classification trees.

A further category of SFs or SPIs can be identified by qualitative measures by comparing the percentage feature presence of an SF or SPI of one group of samples (e.g., samples from diseased subjects) with the percentage feature presence of an SF or SPI in another group of samples (e.g., samples from control subjects). The "percentage feature presence" of an SF or SPI is the percentage of samples in a group of samples in which the SF

or a recombinant nucleic acid which encodes the SPI, a portion of the SPI, or a precursor of the SPI is identified, the gene product can be analyzed. This is achieved by assays based on the physical or functional properties of the product, including radioactive labeling of the product followed by analysis by gel electrophoresis, immunoassay, etc.

The SPIs identified herein can be isolated and purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Alternatively, once a recombinant nucleic acid that encodes the SPI is identified, the entire amino acid sequence of the SPI can be deduced from the nucleotide sequence of the gene coding region contained in the recombinant nucleic acid. As a result, the protein can be synthesized by standard chemical methods known in the art (e.g., see Hunkapiller et al., 1984, Nature 310:105-111).

In another alternative embodiment, native SPIs can be purified from natural sources, by standard methods such as those described above (e.g., immunoaffinity purification).

In a preferred embodiment, SPIs are isolated by the Preferred Technology described supra. For preparative-scale runs, a narrow-range "zoom gel" having a pH range of 2 pH units or less is preferred for the isoelectric step, according to the method described in Westermeier, 1993, Electrophoresis in Practice (VCH, Weinheim, Germany), pp. 197-209 (which is incorporated herein by reference in its entirety); this modification permits a larger quantity of a target protein to be loaded onto the gel, and thereby increases the quantity of isolated SPI that can be recovered from the gel. When used in this way for preparative-scale runs, the Preferred Technology typically provides up to 100 ng, and can provide up to 1000 ng, of an isolated SPI in a single run. Those of skill in the art will appreciate that a zoom gel can be used in any separation strategy that employs gel isoelectric focusing.

The invention thus provides an isolated SPI, an isolated SPI-related polypeptide, and an isolated derivative or fragment of an SPI or an SPI-related polypeptide; any of the foregoing can be produced by recombinant DNA techniques or by chemical synthetic methods.

#### 5.6 Isolation of DNA Encoding an SPI

Specific embodiments for the cloning of a gene encoding an SPI, are presented below by way of example and not of limitation.

The nucleotide sequences of the present invention, including DNA and RNA, and comprising a sequence encoding an SPI or a fragment thereof, or an SPI-related polypeptide, may be synthesized using methods known in the art, such as using conventional chemical approaches or polymerase chain reaction (PCR) amplification. The nucleotide sequences of

the present invention also permit the identification and cloning of the gene encoding an SPI homolog or SPI ortholog including, for example, by screening cDNA libraries, genomic libraries or expression libraries.

For example, to clone a gene encoding an SPI by PCR techniques, anchored degenerate oligonucleotides (or a set of most likely oligonucleotides) can be designed for all SPI peptide fragments identified as part of the same protein. PCR reactions under a variety of conditions can be performed with relevant cDNA and genomic DNAs (e.g., from brain tissue or from cells of the immune system) from one or more species. Also vectorette reactions can be performed on any available cDNA and genomic DNA using the oligonucleotides (which preferably are nested) as above. Vectorette PCR is a method that enables the amplification of specific DNA fragments in situations where the sequence of only one primer is known. Thus, it extends the application of PCR to stretches of DNA where the sequence information is only available at one end. (Arnold C, *PCR Methods Appl* (1991) 1(1):39-42; Dye K.D, *Biotechniques*, (1993) 19(4):550-2). Vectorette PCR may be performed with probes that are, for example, anchored degenerate oligonucleotides (or most likely oligonucleotides) coding for SPI peptide fragments, using as a template a genomic library or cDNA library pools.

Anchored degenerate oligonucleotides (and most likely oligonucleotides) can be designed for all SPI peptide fragments. These oligonucleotides may be labelled and hybridized to filters containing cDNA and genomic DNA libraries. Oligonucleotides to different peptides from the same protein will often identify the same members of the library. The cDNA and genomic DNA libraries may be obtained from any suitable or desired mammalian species, for example from humans.

Nucleotide sequences comprising a nucleotide sequence encoding an SPI or SPI fragment of the present invention are useful for their ability to hybridize selectively with complementary stretches of genes encoding other proteins. Depending on the application, a variety of hybridization conditions may be employed to obtain nucleotide sequences at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical, or 100% identical, to the sequence of a nucleotide encoding an SPI.

For a high degree of selectivity, relatively stringent conditions are used to form the duplexes, such as low salt or high temperature conditions. As used herein, "highly stringent conditions" means hybridization to filter-bound DNA in 0.5 M NaHPO<sub>4</sub>, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65 °C, and washing in 0.1xSSC/0.1% SDS at 68 °C (Ausubel F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3; incorporated herein by reference in its entirety.) For some applications, less stringent conditions for duplex formation are required. As used herein "moderately stringent conditions" means washing in 0.2xSSC/0.1% SDS at 42 °C (Ausubel et al., 1989, *supra*). Hybridization conditions can also

be rendered more stringent by the addition of increasing amounts of formamide, to destabilize the hybrid duplex. Thus, particular hybridization conditions can be readily manipulated, and will generally be chosen depending on the desired results. In general, convenient hybridization temperatures in the presence of 50% formamide are: 42 °C for a probe which is 95 to 100% identical to the fragment of a gene encoding an SPI, 37 °C for 90 to 95% identity and 32 °C for 70 to 90% identity.

In the preparation of genomic libraries, DNA fragments are generated, some of which will encode parts or the whole of an SPI. Any suitable method for preparing DNA fragments may be used in the present invention. For example, the DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNase in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The DNA fragments can then be separated according to size by standard techniques including but not limited to agarose and polyacrylamide gel electrophoresis, column chromatography and sucrose gradient centrifugation. The DNA fragments can then be inserted into suitable vectors, including but not limited to plasmids, cosmids, bacteriophages lambda or T4, and yeast artificial chromosome (YAC). (See, e.g., Sambrook et al, 1989, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K. Vol. I, II; Ausubel F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York). The genomic library may be screened by nucleic acid hybridization to labelled probe (Benton and Davis, *Science* (1977) 196:180; Grunstein and Hogness, *Proc. Natl. Acad. Sci. USA* (1975) 72:3961).

Based on the present description, the genomic libraries may be screened with labelled degenerate oligonucleotide probes corresponding to the amino acid sequence of any peptide of the SPI using optimal approaches well known in the art. Any probe used is at least 10 nucleotides, at least 15 nucleotides, at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, or at least 100 nucleotides. Preferably a probe is 10 nucleotides or longer, and more preferably 15 nucleotides or longer.

In Tables IV and V above, some SPIs disclosed herein were found to correspond to isoforms of previously identified proteins encoded by genes whose sequences are publicly known. (Sequence analysis and protein identification of SPIs was carried out using the methods described in Section 6.1.14). To screen such a gene, any probe may be used that is complementary to the gene or its complement; preferably the probe is 10 nucleotides or longer, more preferably 15 nucleotides or longer. The SWISS-PROT and trEMBL databases

(held by the Swiss Institute of Bioinformatics (SIB) and the European Bioinformatics Institute (EBI) which are available at <http://www.ebi.ac.uk>) and the GenBank database (held by the National Institute of Health (NIH) which is available at <http://www.ncbi.nlm.nih.gov/GenBank/>) provide protein sequences for the SPIs listed in Tables IV and V under the following accession numbers and each sequence is incorporated herein by reference:

Table X. Nucleotide sequences encoding SPIs, SPI Related Proteins or ERPIs

SF#	SPI#	Accession Numbers of Identified Sequences
SF-14	SPI-6	P10643
SF-16	SPI-231	P05090
SF-19	SPI-312	P41222
SF-20	SPI-352	6049608 (gb)
SF-21	SPI-232	P48668
SF-22	SPI-7	P41222
SF-24	SPI-353	P01034
SF-24'	SPI-354	P20472
SF-27	SPI-233	P01034
SF-28	SPI-8	P01034
SF-29	SPI-9	P01034
SF-30	SPI-10	P05067
SF-31	SPI-11	Q99435
SF-32	SPI-13	P36955
SF-32	SPI-234	P02679
SF-33	SPI-355	P05067
SF-33	SPI-356	P05155
SF-35	SPI-15	P02649
SF-35	SPI-16	P10909
SF-36	SPI-17	P41222
SF-37	SPI-18	P06396
SF-38	SPI-19	P36955
SF-38	SPI-235	P35747
SF-38	SPI-236	4240271 (gb)
SF-39	SPI-357	P01028

SF-40	SPI-20	Q92876
SF-41	SPI-21	P36955
SF-42	SPI-23	P06396
SF-43	SPI-26	S802984 (gb)
SF-43	SPI-24	P36955
SF-43	SPI-25	P04469
SF-44	SPI-28	P41222
SF-45	SPI-29	P36955
SF-45	SPI-30	5802984 (gb)
SF-46	SPI-32	P01024
SF-47	SPI-33	P01024
SF-48	SPI-34	P10909
SF-48	SPI-35	P02649
SF-49	SPI-36	P41222
SF-51	SPI-38	P41222
SF-52	SPI-39	5802984 (gb)
SF-53	SPI-237	P05067
SF-55	SPI-238	NOVEL
SF-55	SPI-239	P80108
SF-55	SPI-41	P01019
SF-55	SPI-240	NOVEL
SF-56	SPI-42	P01008
SF-57	SPI-43	P05452
SF-58	SPI-241	P41222
SF-58	SPI-44	P30088
SF-81	SPI-321	P01028
SF-81	SPI-322	P41222
SF-82	SPI-323	P01028
SF-83	SPI-54	P02671
SF-84	SPI-381	P02649
SF-85	SPI-382	P41222
SF-86	SPI-56	P35908
SF-87	SPI-383	P41222

SF-87	SPI-384	P36955
SF-88	SPI-57	P01028
SF-90	SPI-324	P01027
SF-91	SPI-325	P02649
SF-92	SPI-326	P13645
SF-93	SPI-359	P02024
SF-93	SPI-360	1095700.4 (gb)
SF-94	SPI-58	P05155
SF-96	SPI-361	P02780
SF-97	SPI-327	P01024
SF-98	SPI-362	P02768
SF-99	SPI-328	P00751
SF-100	SPI-242	P02849
SF-101	SPI-329	P01024
SF-102	SPI-59	P36955
SF-102	SPI-60	P02649
SF-107	SPI-243	106655 (gb)
SF-107	SPI-330	106655 (gb)
SF-108	SPI-244	P01028
SF-111	SPI-62	P01034
SF-112	SPI-331	P41222
SF-114	SPI-332	P36955
SF-115	SPI-63	P02783
SF-118	SPI-65	P01877
SF-117	SPI-333	1381979 (gb)
SF-118	SPI-334	P02768
SF-123	SPI-335	662280 (gb)
SF-124	SPI-385	2647262 (gb)
SF-126	SPI-245	P02538
SF-132	SPI-336	P14625
SF-135	SPI-67	P01028
SF-143	SPI-337	P06732
SF-144	SPI-363	P02023

SF-151	SPI-69	P36955
SF-153	SPI-338	Q15668
SF-153	SPI-339	P01024
SF-154	SPI-365	P02023
SF-157	SPI-340	P41222
SF-158	SPI-387	P41222
SF-158	SPI-388	8368450 (gb)
SF-159	SPI-73	P02649
SF-160	SPI-74	P01028
SF-161	SPI-75	P02649
SF-163	SPI-78	P02649
SF-164	SPI-77	P01028
SF-165	SPI-389	P36222
SF-166	SPI-246	P10909
SF-167	SPI-78	P02649
SF-168	SPI-390	P36955
SF-169	SPI-391	Q13827
SF-170	SPI-80	P02649
SF-171	SPI-392	P41222
SF-172	SPI-393	Q13827
SF-173	SPI-247	401767 (gb)
SF-173	SPI-81	P15169
SF-174	SPI-82	P02768
SF-176	SPI-83	P36955
SF-178	SPI-248	7435109 (gb)
SF-178	SPI-249	P05156
SF-177	SPI-85	P02750
SF-178	SPI-250	P01027
SF-179	SPI-87	P36955
SF-180	SPI-251	892271 (gb)
SF-181	SPI-88	P04220
SF-182	SPI-252	1942472 (gb)
SF-184	SPI-253	1402890 (gb)

SF-188	SPI-254	P01028
SF-187	SPI-255	P01028
SF-188	SPI-394	P35908
SF-189	SPI-91	P02790
SF-190	SPI-257	P23144
SF-190	SPI-258	P08697
SF-191	SPI-92	P01024
SF-191	SPI-259	P02749
SF-194	SPI-261	P01024
SF-196	SPI-262	P08603
SF-197	SPI-85	P41222
SF-197	SPI-93	1942471 (gb)
SF-198	SPI-96	P06398
SF-199	SPI-97	P10643
SF-200	SPI-99	P13671
SF-201	SPI-100	P29622
SF-202	SPI-101	P06868
SF-209	SPI-105	P01344
SF-211	SPI-367	Q92876
SF-212	SPI-267	P08603
SF-213	SPI-264	P10909
SF-213	SPI-107	P01028
SF-215	SPI-341	P08603
SF-217	SPI-113	1942472 (gb)
SF-219	SPI-114	P16035
SF-221	SPI-342	P06396
SF-222	SPI-115	P10643
SF-222	SPI-265	P02768
SF-223	SPI-118	Q15818
SF-226	SPI-268	P51693
SF-226	SPI-267	P08571
SF-227	SPI-268	4758978 (gb)
SF-227	SPI-269	P29622

SF-228	SPI-270	P01027
SF-229	SPI-122	P01024
SF-230	SPI-123	7019363 (gb)
SF-231	SPI-124	P07358
SF-232	SPI-343	P01024
SF-233	SPI-344	2745741 (gb)
SF-233	SPI-345	P01878
SF-235	SPI-346	P02780
SF-238	SPI-127	P00747
SF-242	SPI-129	P04284
SF-243	SPI-130	P02675
SF-243	SPI-273	P05154
SF-244	SPI-369	P02023
SF-248	SPI-370	P01034
SF-248	SPI-274	P08603
SF-250	SPI-133	P28340
SF-250	SPI-132	P02649
SF-255	SPI-138	Q02246
SF-257	SPI-275	P01876
SF-258	SPI-139	P08571
SF-262	SPI-397	P06396
SF-264	SPI-141	Q12860
SF-265	SPI-142	Q02246
SF-267	SPI-143	P02571
SF-268	SPI-151	Q02248
SF-269	SPI-152	P02790
SF-270	SPI-153	P01028
SF-271	SPI-154	P02788
SF-272	SPI-155	P08697
SF-273	SPI-348	P10643
SF-280	SPI-164	P01028
SF-282	SPI-166	Q14112
SF-283	SPI-167	P01876

SF-286	SPI-169	P01043
SF-286	SPI-170	P01876
SF-289	SPI-398	P08603
SF-291	SPI-176	P36955
SF-291	SPI-175	P01028
SF-292	SPI-349	P06733
SF-293	SPI-372	P04406
SF-296	SPI-278	P08603
SF-300	SPI-179	Q02246
SF-300	SPI-281	6753222 (gb)
SF-301	SPI-375	P01024
SF-302	SPI-376	P01024
SF-303	SPI-181	P17174
SF-304	SPI-182	Q15582
SF-306	SPI-399	Q15394
SF-307	SPI-183	P02790
SF-309	SPI-185	P02790
SF-309	SPI-184	P04217
SF-317	SPI-400	P19021
SF-320	SPI-189	P41222
SF-321	SPI-379	P00751
SF-322	SPI-190	Q03591
SF-324	SPI-193	P06727
SF-326	SPI-285	P04217
SF-327	SPI-195	7341255 (gb)
SF-332	SPI-289	P01028
SF-333	SPI-200	P02750
SF-336	SPI-290	P00747
SF-340	SPI-205	P01028
SF-342	SPI-206	NOVEL (AL008583)
SF-344	SPI-296	P17900
SF-344	SPI-297	P41222
SF-348	SPI-211	P02790

SF-348	SPI-302	6518913 (gb)
SF-349	SPI-303	P05201
SF-352	SPI-213	P30086
SF-352	SPI-214	P41222
SF-354	SPI-308	P40252
SF-368	SPI-401	P10643
SF-368	SPI-402	4507721 (gb)
SF-369	SPI-403	P01023
SF-370	SPI-404	P01024
SF-372	SPI-405	P01034
SF-373	SPI-406	1743885 (gb)
SF-373	SPI-407	P06396
SF-376	SPI-408	P13645
SF-376	SPI-409	P18065
SF-379	SPI-410	P02549
SF-380	SPI-411	P01034
SF-382	SPI-412	P01024
SF-389	SPI-413	P02647
SF-391	SPI-414	P05090
SF-393	SPI-415	P41222
SF-396	SPI-416	P02649
SF-396	SPI-417	P41222
SF-397	SPI-418	P01034
SF-398	SPI-419	P41222
SF-399	SPI-420	P01034
SF-402	SPI-421	P02766
SF-404	SPI-422	P05090
SF-405	SPI-423	P01876
SF-406	SPI-424	P01024
SF-406	SPI-425	P10909
SF-407	SPI-426	P01034
SF-409	SPI-427	P04264
SF-409	SPI-428	7662374

SF-410	SPI-429	P08697
SF-410	SPI-430	P02748
SF-410	SPI-431	P01876
SF-411	SPI-432	P07360
SF-412	SPI-433	P10909
SF-412	SPI-434	4240149 (gb)
SF-414	SPI-435	P08697
SF-416	SPI-436	P02774
SF-416	SPI-437	436857.2
SF-416	SPI-438	P01019
SF-416	SPI-439	177636 (gb)
SF-417	SPI-440	410564
SF-420	SPI-441	P05090
SF-421	SPI-442	P54289
SF-422	SPI-443	P01019
SF-423	SPI-444	6651381
SF-424	SPI-445	P08603
SF-425	SPI-446	P01034
SF-434	SPI-447	AH026519.1
SF-440	SPI-448	P02671
SF-443	SPI-449	P02763
SF-446	SPI-450	2745741
SF-448	SPI-451	B918224
SF-451	SPI-452	P01034
SF-459	SPI-453	P13645
SF-462	SPI-454	P02766
SF-462	SPI-455	237026.3
SF-464	SPI-456	P41222
SF-471	SPI-457	P02649
SF-472	SPI-458	10835792
SF-472	SPI-459	P41222
SF-475	SPI-460	P04104
SF-477	SPI-461	P01024

SF-478	SPI-462	P01028
SF-487	SPI-463	1096891 (gb)
SF-494	SPI-464	Q03591
SF-496	SPI-465	P00747
SF-496	SPI-466	1160616
SF-502	SPI-467 <sub>a</sub>	P02763
ERF-2	ERPI-1	P41222
ERF-2	ERPI-2	P06396

For each of the following SPIs: SPI-206, SPI-238 and SPI-240, the partial sequence information derived from tandem mass spectrometry was not found to be described as a transcribed protein in any known public database. SPI-206, SPI-238 and SPI-240 are therefore listed as 'NOVEL' in Table X. SPI-206, SPI-238 and SPI-240 have been cloned, and are further described below. For any SPI, degenerate probes, or probes taken from the sequences described above by accession number may be used for screening. In the case of degenerate probes, they can be constructed from the partial amino sequence information obtained from tandem mass spectra of tryptic digest peptides of the SPI. To screen such a gene, any probe may be used that is complementary to the gene or its complement; preferably the probe is 10 nucleotides or longer, more preferably 15 nucleotides or longer. When a library is screened, clones with insert DNA encoding the SPI or a fragment thereof will hybridize to one or more members of the corresponding set of degenerate oligonucleotide probes (or their complement). Hybridization of such oligonucleotide probes to genomic libraries is carried out using methods known in the art. For example, hybridization with one of the above-mentioned degenerate sets of oligonucleotide probes, or their complement (or with any member of such a set, or its complement) can be performed under highly stringent or moderately stringent conditions as defined above, or can be carried out in 2X SSC, 1.0% SDS at 50 °C and washed using the washing conditions described supra for highly stringent or moderately stringent hybridization.

In yet another aspect of the invention, clones containing nucleotide sequences encoding the entire SPI, a fragment of an SPI, an SPI-related polypeptide, or a fragment of an SPI-related polypeptide any of the foregoing may also be obtained by screening expression libraries. For example, DNA from the relevant source is isolated and random fragments are prepared and ligated into an expression vector (e.g., a bacteriophage, plasmid, phagemid or cosmid) such that the inserted sequence in the vector is capable of being expressed by the host cell into which the vector is then introduced. Various screening assays can then be used to select for the expressed SPI or SPI-related polypeptides. In one embodiment, the various anti-

SPI antibodies of the invention can be used to identify the desired clones using methods known in the art. See, for example, Harlow and Lane, 1988 *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Appendix IV. Colonies or plaques from the library are brought into contact with the antibodies to identify those clones that bind antibody.

In an embodiment, colonies or plaques containing DNA that encodes an SPI, a fragment of an SPI, an SPI-related polypeptide, or a fragment of an SPI-related polypeptide can be detected using DYNA Beads according to Olsvick et al, 29th ICAAC, Houston, Tex. 1989, incorporated herein by reference. Anti-SPI antibodies are crosslinked to tosylated DYNA Beads M280, and these antibody-containing beads are then contacted with colonies or plaques expressing recombinant polypeptides. Colonies or plaques expressing an SPI or SPI-related polypeptide are identified as any of those that bind the beads.

Alternatively, the anti-SPI antibodies can be nonspecifically immobilized to a suitable support, such as silica or Celite® resin. This material is then used to adsorb to bacterial colonies expressing the SPI protein or SPI-related polypeptide as described herein.

In another aspect, PCR amplification may be used to isolate from genomic DNA a substantially pure DNA (*i.e.*, a DNA substantially free of contaminating nucleic acids) encoding the entire SPI or a part thereof. Preferably such a DNA is at least 95% pure, more preferably at least 99% pure. Oligonucleotide sequences, degenerate or otherwise, that correspond to peptide sequences of SPIs disclosed herein can be used as primers.

PCR can be carried out, *e.g.*, by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp® or AmpliTaq DNA polymerase). One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between the degenerate primers and the corresponding sequences in the DNA. After successful amplification of a segment of the sequence encoding an SPI, that segment may be molecularly cloned and sequenced, and utilized as a probe to isolate a complete genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*.

The gene encoding an SPI can also be identified by mRNA selection by nucleic acid hybridization followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified DNA encoding an SPI of another species (*e.g.*, mouse, human). Immunoprecipitation analysis or functional assays (*e.g.*, aggregation ability *in vitro*; binding to receptor) of the *in vitro* translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In

addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized antibodies that specifically recognize an SPI. A radiolabelled cDNA encoding an SPI can be synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabelled mRNA or cDNA may then be used as a probe to identify the DNA fragments encoding an SPI from among other genomic DNA fragments.

Alternatives to isolating genomic DNA encoding an SPI include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA that encodes the SPI. For example, RNA for cDNA cloning of the gene encoding an SPI can be isolated from cells that express the SPI. Those skilled in the art will understand from the present description that other methods may be used and are within the scope of the invention.

Any suitable eukaryotic cell can serve as the nucleic acid source for the molecular cloning of the gene encoding an SPI. The nucleic acid sequences encoding the SPI can be isolated from vertebrate, mammalian, primate, human, porcine, bovine, feline, avian, equine, canine or murine sources. The DNA may be obtained by standard procedures known in the art from cloned DNA (*e.g.*, a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell. (See, *e.g.*, Sambrook et al, 1989, *Molecular Cloning: A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (*ed.*), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences.

The identified and isolated gene or cDNA can then be inserted into any suitable cloning vector. A large number of vector-host systems known in the art may be used. As those skilled in the art will appreciate, the only limitation is that the vector system chosen be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, plasmids such as PBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene) or modified viruses such as adenoviruses, adeno-associated viruses or retroviruses. The insertion into a cloning vector can be accomplished, for example, by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleic acid sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific, chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and the gene encoding an SPI may be modified by homopolymeric tailing. Recombinant molecules can be introduced

into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated gene encoding the SPI, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The nucleotide sequences of the present invention include nucleotide sequences encoding amino acid sequences with substantially the same amino acid sequences as native SPIs, nucleotide sequences encoding amino acid sequences with functionally equivalent amino acids, nucleotide sequences encoding SPIs, a fragments of SPIs, SPI-related polypeptides, or fragments of SPI-related polypeptides.

In a specific embodiment, an isolated nucleic acid molecule encoding an SPI-related polypeptide can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of an SPI such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Standard techniques known to those of skill in the art can be used to introduce mutations, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed and the activity of the protein can be determined.

### 5.6.1 Cloning and Characterization of SPI-206

SPI-206 was isolated, subjected to proteolysis, and analyzed by mass spectrometry using the methods and apparatus of the Preferred Technology. Using the SEQUEST search

program as described in the Examples, *infra*, uninterpreted tandem mass spectra of tryptic digest peptides were searched against a database of public domain proteins constructed of protein entries in the non-redundant database held by the National Centre for Biotechnology Information (NCBI) which is accessible at <http://www.ncbi.nlm.nih.gov>. As a result of database searching, the following amino acid sequence of a tryptic digest peptide of SPI-206 was determined from a match to a tryptic digest peptide found in a translation of a human DNA sequence (protein ID CAA15430.1, accessible at <http://www.ncbi.nlm.nih.gov/entrez/>): ELDVVLQGR (shown in Figure 2).

In cases where no amino acid sequences could be determined through searching using the SEQUEST program, tandem mass spectra of the peptides were interpreted manually, using methods known in the art as described in the Examples, *infra*. In the method of tandem mass spectrometry used for sequencing peptides in the present invention, the following pairs of amino acids could not be distinguished from each other: leucine and isoleucine; and, under certain circumstances, glutamine and lysine, and phenylalanine and oxidized methionine. As used herein, an amino acid sequence "as determined by mass spectrometry" refers to the set of amino acid sequences containing at the indicated positions, one or other member of the designated pairs of amino acids. For example, the amino acid sequence P(L/I)A indicates the amino acid sequences PLA and PIA. As will be obvious to one of skill in the art, a sequence containing n designated pairs indicates 2<sup>n</sup> amino acid sequences. In Table XI, the possible amino acid sequence is listed for a single sequence determined by mass spectrometry.

Table XI Partial Amino Acid Sequences of SPI-206

SP#	SP#	Mass of peptide analyzed by mass spectrometry*	Partial amino acid sequences determined	Mass to N-terminus	Mass to C-terminus	pI	MW
SF-342	SPI-206	1569.9	[M]R[Q]L[G]	283.27	675.35	6.08	29463

\*In Table XI, the masses determined by mass spectrometry have an error of mass measurement of 100 parts-per-million (ppm) or less. For a given measured mass, M, having an error of mass measurement of a ppm, the error of mass measurement can be calculated as  $(M \times 10^{-6}) / (100 + 100000)$ .

As used herein, the "mass of the peptide analyzed by mass spectrometry" is the mass of the singly protonated peptide measured by mass spectrometry, and corresponds to the total mass of the constituent amino acid residues of the peptide with the addition of a water molecule ( $H_2O$ ) and a single proton ( $H^+$ ). As used herein, the "mass to the N-terminus" corresponds to the total mass of the constituent amino acid residues extending from the start of the partial sequence to the N-terminus of the peptide. As used herein, the "mass to the C-terminus" corresponds to the total mass of the constituent amino acid residues extending from the end of the partial sequence to the C-terminus of the peptide with the addition of a water molecule ( $H_2O$ ), and a single proton ( $H^+$ ).

The partial amino acid sequence and masses listed in Table XI were found to correspond to a peptide within the same putative human protein identified using SEQUEST, (i.e. protein ID CAA15430.1, accessible at <http://www.ncbi.nlm.nih.gov/entrez>). The full amino acid sequence of the peptide listed in Table XI as a result of the match was found to be:

5 : GILILGQEQDITLGGR (shown in Figure 2).

The DNA sequences encoding the two identified peptides are as follows:

E L D V L Q G R  
gag ttg gac gtc ctg cag ggt cgt

10 G I L I L G Q E Q D T L G G R  
ggg atc ctt atc ttg ggc cag gag gat acc ctg ggt ggc cgg

The human protein of database accession number CAA15430.1, the neuronal pentraxin receptor, whose gene is located on chromosome 22q12.3-13.2 (accession ID AL008583), is an ortholog of rat neuronal pentraxin receptor (Kirkpatrick LL, Matzuk MM, Dodds DC, Perin MS. Biochemical interactions of the neuronal pentraxins. Neuronal pentraxin (NP) receptor binds to taipoxin and taipoxin-associated calcium-binding protein 49 via NP1 and NP2. *J Biol Chem.* (2000) Jun 9;275(23):17786-92, Dodds DC, Omelia IA, Cushman SJ, Helms JA, Perin MS. Neuronal pentraxin receptor (NPR), a novel putative integral membrane pentraxin that interacts with neuronal pentraxin 1 and 2 and taipoxin-associated calcium-binding protein 49. *J Biol Chem.* (1997) Aug 22;272(34):21488-94).

A nucleotide sequence (Figure 2B) encoding a peptide (Figure 2A) comprising the above two peptides can be cloned by using the following primers:

25 5' cgccatcacgtgaaggctctg 3'  
5' ctggatgaggatggccctcatgc 3'

Primers useful for determining the sequence of the nucleotide sequence are:

30 5' tgttcagccgccttctgtgcac 3'  
5' tcttagcagtacaatctcggttgg 3'

The peptide of Figure 2A has 90% homology with the rat polypeptide (AAB62885).  
35 The rat protein, a putative integral membrane pentraxin, has 49 and 48% identity to neuronal pentraxin 1 (NP1) and neuronal pentraxin 2 (NP2), respectively (Dodds DC et al., *supra*; Hsu, et al., 1995, *Genomics* 28:2, 220-227). These proteins are suggested to be constituents of a

pathway involved in the clearance of synaptic debris. Addition of NP1 to glial cultures renders them susceptible to a neurotoxin toxicity (Dodds DC et al., *supra*). NP1 is expressed on the cell membrane and can form heteropentamers with NP1 and NP2 that can be released from cell membranes (Kirkpatrick et al., *supra*).

- 5 NP1 has homology to previously identified pentraxins, such as serum amyloid P protein (Dodds DC et al., *supra*). Serum amyloid P protein has been widely described for its role in amyloid (Botto M, et al., *Nat Med.* 1997 Aug;3(8):855-9; International Patent Publication WO9505394), and in particular its implication in Alzheimer disease progression (Tremont GA, Lovell LB, Pepys MB. Serum amyloid P component prevents proteolysis of the amyloid fibrils of Alzheimer disease and systemic amyloidosis. *Proc Natl Acad Sci U S A.* 1995 May 9;92(10):4299-303) and diagnostic (Hawkins PN, Rossor MN, Gallimore JR, Miller B, Moore BG, Pepys MB. Concentration of serum amyloid P component in the CSF as a possible marker of cerebral amyloid deposits in Alzheimer's disease. *Biochem Biophys Res Commun.* 1994 Jun 15;210(2):722-6) as well as other degenerative disorders (Kalaria RN, Galloway PG, Perry G. Widespread serum amyloid P immunoreactivity in cortical amyloid deposits and the neurofibrillary pathology of Alzheimer's disease and other degenerative disorders. *Neuropathol Appl Neurobiol.* 1991 Jun;17(3):189-201), and cerebral cell death (Urbanyi Z, Lakics V, Erdi SL. Serum amyloid P component-induced cell death in primary cultures of rat cerebral cortex. *Eur J Pharmacol.* 1994 Aug 3;270(4):375-8).

- 20 The peptide of Figure 2A also exhibits homology to neuronal-activity-regulated pentraxin (NARP). See International Patent Publication WO9739133.

Therefore the peptide of Figure 2A, which has 48% homology with serum amyloid P, and which is increased by 1.92 fold in Schizophrenia-affected patients, can be used for screening diagnosis, prognosis of Schizophrenia according to the methods of the invention.

- 25 Any of the activities described in the above references concerning amyloid P, NARP, NPF, NP1 and NP2 can be used as the basis for functional assays for compounds capable of inhibiting or stimulating the relevant activity of the peptide of Figure 2A. In addition, compounds capable of modulating the activities of amyloid P, NARP, NPF, NP1 and NP2 are candidate compounds for the treatment of Alzheimer's disease. Thus, assays for such 30 compounds may also be performed, by, for example, recombinantly expressing amyloid P, NARP, NPF, NP1 or NP2 and testing for compounds capable of modulating the activity of the expressed protein.

The peptide of Figure 2A may be recombinantly expressed by constructing an expression vector comprising the nucleic acid sequence of Figure 2B, or portions thereof.

- 35 The expressed recombinant protein may be used in assays for compounds capable of modulating the activity of the recombinant protein. In addition, assays for compounds capable of inhibiting or stimulating the cleavage of the peptide of Figure 2A may be

performed. In a particular embodiment, only a portion of the peptide is recombinantly produced. In a preferred embodiment, the portion of the peptide produced comprise a cleavage site. In a preferred embodiment, the portion of the peptide comprises amino acid residues 26 to 46, or residues 237 to 247. In another preferred embodiment, a truncated peptide comprising or consisting of the carboxyl terminus is produced, e.g., from amino acid residue 28, 36, 237, 243, 264 or 327 to the carboxyl terminus of the peptide.

#### 5.6.2 Cloning and Characterization of SPI-238 and SPI-240

SPI-238 and SPI-240 were each isolated, subjected to proteolysis, and analyzed by mass spectrometry using the methods and apparatus of the Preferred Technology. Using the SEQUEST search program as described in the Examples, *infra*, uninterpreted tandem mass spectra of tryptic digest peptides were searched against a database of public domain proteins constructed of protein entries in the non-redundant database held by the National Centre for Biotechnology Information (NCBI) which is accessible at <http://www.ncbi.nlm.nih.gov/> and also constructed of Expressed Sequence Tags entries (<http://www.ncbi.nlm.nih.gov/dbEST/index.html>). As a result of database searching, the following amino acid sequence of a tryptic digest peptide of both SPI-238 and SPI-240 were determined from a match to a tryptic digest peptide in a conceptual translation of EST AA326679: EWVVAIESDSVQPVPR (shown in Figure 4B).

In cases where no amino acid sequences could be determined through searching using the SEQUEST program, tandem mass spectra of the peptides were interpreted manually, using methods known in the art as described in the Examples, *infra*. In the method of tandem mass spectrometry used for sequencing peptides in the present invention, the following pairs of amino acids could not be distinguished from each other: leucine and isoleucine; and, under certain circumstances, glutamine and lysine, and phenylalanine and oxidized methionine. As used herein, an amino acid sequence "as determined by mass spectrometry" refers to the set of amino acid sequences containing at the indicated positions, one or other member of the designated pairs of amino acids. For example, the amino acid sequence P[LA] indicates the amino acid sequences PLA and PIA. As will be obvious to one of skill in the art, a sequence containing n designated pairs indicates  $2^n$  amino acid sequences. For both SPI-238 and SPI-240 the same partial sequence was determined by mass spectrometry and is listed in Table XII.

Table XII. Partial Amino Acid Sequences of SPI-238 and SPI-240 as Determined by Mass Spectrometry

SF#	SPI#	Mass of peptide analyzed by mass spectrometry*	Partial amino acid sequences	Mass to N-terminus	Mass to C-terminus	pI	MW
SF-55	SPI-238	1258.65	H[L/I/D][L/J]EEYR	184.07	0.00	4.94	59288
SF-66	SPI-240	1258.65	H[L/I/D][L/J]EEYR	184.07	0.00	5.04	57690

\*The masses determined by mass spectrometry have an error of mass measurement of 100 parts-per-million (ppm) or less. For a given measured mass,  $M$ , having an error of mass measurement of  $\pm z$  ppm, the error of mass measurement can be calculated as  $(M \times z \times 1000000)$ .

- 5 As used herein, the "mass of the peptide analyzed by mass spectrometry" is the mass of the singly protonated peptide measured by mass spectrometry, and corresponds to the total mass of the constituent amino acid residues of the peptide with the addition of a water molecule ( $H_2O$ ) and a single proton ( $H^+$ ). As used herein, the "mass to N-terminus" 10 corresponds to the total mass of the constituent amino acid residues extending from the start of the partial sequence to the N-terminus of the peptide. As used herein, the "mass to C-terminus" corresponds to the total mass of the constituent amino acid residues extending from the end of the partial sequence to the C-terminus of the peptide with the addition of a water molecular ( $H_2O$ ) and a single proton ( $H^+$ ).

- 15 The partial amino acid sequence and masses listed in Table XII were not found to match to any sequences in the database used.

- EST AA326679 shows 44% amino acid identity with a putative human protein derived from a conceptual translation of the cDNA CAB07646.1 (available at <http://www.ncbi.nlm.nih.gov/entrez>). The C terminus of this protein sequence (CAB07646.1) shows a similar level of homology with a further brain tissue derived EST (AIS89129) (BLASTN, BLAST, Altschul, Stephen F., Gish, Warren, Miller, Webb, Myers, Eugene W., and Lipman, David J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215: 403-410.). This EST sequence does not overlap with EST AA326679 so that the possibility remained that the partial amino acid sequence and masses listed in Table XII could be encoded by the no-overlapping region of these 2 ESTs.

- Opposing PCR primers (1 & 2 from Table XIII) from EST AA326679 and EST AIS89129 were used in a PCR reaction (1 ml of Advantage 2 cDNA polymerase mix (Clontech) in a buffer containing 50mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl<sub>2</sub>, pH8.3; 0.2mM each of dATP, dCTP, dGTP, dTTP and 10 pmoles of oligonucleotide primers. 20 Reactions were routinely made to a final volume of 50ml and amplification carried out in a PE GeneAmpSystems 9700 PCR machine with the following cycling conditions: initial denaturation of 94 °C for 1 minute followed by 30 cycles of 94 °C for 30 seconds, 55 °C for

30 seconds and 72 °C for 2 minutes. Reaction products were resolved by standard agarose gel electrophoresis and stained with Ethidium Bromide) on 10μg of whole brain cDNA (Clontech, USA). The resulting 1.6kb fragment was purified from primers and buffers (Qiagen, UK) and sequenced using the primers given in Table XIII (1,2,3 & 4). This generated overlapping sequence for the entire product. Analysis of this DNA sequence (GCC, UK) shows a complete ORF now including the partial amino acid sequence and masses listed in Table XIII. Figure 4B shows the DNA sequence. Figure 4A show the protein sequence of the open reading frame (ORF), demonstrating the presence of the two peptides identified by mass spectrometry.

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Table XIII. Primer Sequences

Primer	Name	Sequence (5' --- 3')
1	SPI 238/240 F1	gccttaatggntcccaaactc
2	SPI 238/240 R1	gagggtgaatctgtcagttggatc
3	SPI 238/240 SF	atggaaagggctggcttctgttg
4	SPI 238/240 SR	aaggatgtggtaccccgagg

The DNA sequences encoding the sequences of two identified peptides are as follows:

15 gag tgg gtg gcc atc gag agc gac tct gtc eaq cct gtg cct  
Glu Trp Val Ala Ile Glu Ser Asp Ser Val Gln Pro Val Pro  
and  
20 gcc atc cat cta gac cta gaa gaa tac cgg  
Ala Ile His Leu Asp Leu Glu Glu Tyr Arg

A Blast search against High Throughput Genomic Sequencing data (<http://www.ncbi.nlm.nih.gov/blast>) localized the SPI-238 and SPI-240 sequence (25 EWVVADESDSVQPVPR to chromosome 18 - clone RP11-231E4, map 18 (AC009704).

In a parallel study on Bipolar Affective Disorder (BAD), the protein corresponding to SPI-238 and SPI-240 was also found to be differentially present in samples of CSF from subjects having BAD compared with samples of CSF from subjects free from BAD, being decreased 2.27 fold (in the copending US patent application number 60/254830 which is 30 incorporated herein by reference).

79), the herpes thymidine kinase promoter (Wagner et al, *Proc. Natl. Acad. Sci. USA* (1981) 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al, *Nature* (1982) 296:39-42), the tetracycline (Tet) promoter (Gossen et al, *Proc. Natl. Acad. Sci. USA* (1995) 89:5547-5551); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamroff, et al, *Proc. Natl. Acad. Sci. USA* (1978) 75:3727-3731), or the lac promoter (DeBoer, et al, *Proc. Natl. Acad. Sci. USA* (1983) 80:21-25; see also "Useful proteins from recombinant bacteria" in *Scientific American* (1980) 242:74-94); plant expression vectors comprising the napaline synthase promoter region (Herrera-Estrella et al, *Nature* (1984) 310(5973):115-20) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al, *Nucl. Acids Res.* (1981) 9:2871), and the promoters of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al, *Nature* (1984) 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alkanol dehydrogenase) promoter, PGK (phosphoglyceral kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase 1 gene control region which is active in pancreatic acinar cells (Swift et al, *Cell* (1984) 38:639-646; Ornitz et al, *Cold Spring Harbor Symp. Quant. Biol.* (1986) 50:399-409; MacDonald, *Hepatology* (1987) 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hensman, *Nature* (1985) 315:115-122); immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al, *Cell* (1984) 38:647-658; Adams et al, 1985, *Nature* 318:533-538; Alexander et al, *Mol. Cell. Biol.* (1987) 7:1436-1444); mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al, *Cell* (1986) 45:485-495); albumin gene control region which is active in liver (Pinkert et al, *Genes and Dev.* (1987) 1:268-276); alpha-fetoprotein gene control region which is active in liver (Krumlauf et al, *Mol. Cell. Biol.* (1985) 5:1639-1648; Hammer et al, *Science* (1987) 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al, *Genes and Dev.* (1987) 1:161-171); beta-globin gene control region which is active in myeloid cells (Mogram et al, *Nature* (1985) 315:338-340; Kolllias et al, *Cell* (1986) 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al, *Cell* (1987) 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sami, *Nature* (1985) 314:283-286); neuronal-specific enolase (NSE) which is active in neuronal cells (Mardili et al, *Gen. Virol.* (1999) 80:571-83); brain-derived neurotrophic factor (BDNF) gene control region which is active in neuronal cells (Tabuchi et al, *Biochem. Biophys. Res. Com.* (1998) 233:818-823); glial fibrillary acidic protein (GFAP) promoter which is active in astrocytes (Gomes et al, *Bras J Med Biol Res* (1999) 32(5):619-631; Mardili et al, *Gen. Virol.* (1999) 80:571-83) and gonadotropin-releasing hormone gene

The patent WO99/58660 disclosed 97 human secreted proteins. These included a sequence, identified as Gene No: 21, which corresponds to SPI-238 and SPI-240 discussed herein. However, this disclosure did not provide any isolated protein, nor did it identify SPI 238/240 as being differentially present in samples of CSF from subjects having BAD 5 compared with a sample of CSF subjects free from BAD and in samples of CSF from subjects having Schizophrenia compared with a sample of CSF subjects free from Schizophrenia.

### 5.7 Expression of DNA Encoding SPIs

The nucleotide sequence coding for an SPI, an SPI analog, an SPI-related peptide, or a fragment or other derivative of any of the foregoing, can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native gene encoding the SPI or its flanking 15 regions, or the native gene encoding the SPI-related polypeptide or its flanking regions. A variety of host-vector systems may be utilized in the present invention to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed 20 with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In specific embodiments, a nucleotide sequence encoding a human gene (or a nucleotide sequence encoding a functionally active portion of a human SPI) is expressed. In yet another 25 embodiment, a fragment of an SPI comprising a domain of the SPI is expressed.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional and translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* 30 recombinant (genetic recombination). Expression of nucleic acid sequence encoding an SPI or fragment thereof may be regulated by a second nucleic acid sequence so that the SPI or fragment is expressed in a host transformed with the recombinant DNA molecule. For example, expression of an SPI may be controlled by any promoter or enhancer element known in the art. Promoters which may be used to control the expression of the gene 35 encoding an SPI or an SPI-related polypeptide include, but are not limited to, the SV40 early promoter region (Benoist and Chambon *Nature* (1981) 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al, *Cell* (1980) 22:787-

control region which is active in the hypothalamus (Mason et al, *Science* (1986) 234:1372-1378).

In a specific embodiment, a vector is used that comprises a promoter operably linked to an SPI-encoding nucleic acid, one or more origins of replication, and, optionally, one or 5 more selectable markers (e.g., an antibiotic resistance gene).

In a specific embodiment, an expression construct is made by subcloning an SPI or an SPI-related polypeptide coding sequence into the EcoRI restriction site of each of the three pGEX vectors (Ghoshthoma S-Transferase expression vectors; Smith and Johnson, *Gene* (1988) 7:31-40). This allows for the expression of the SPI product or SPI-related polypeptide from the subclone in the correct reading frame.

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the SPI coding sequence or SPI-related polypeptide coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene 15 may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts, (e.g., see Logan & Shiek, *Proc. Natl. Acad. Sci. USA* (1984) 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding 20 sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription 25 enhancer elements, transcription terminators, etc. (see Bitner et al, *Methods Enzymol.* (1987) 153:51-544).

Expression vectors containing inserts of a gene encoding an SPI or an SPI-related polypeptide can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In 30 the first approach, the presence of a gene encoding an SPI inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted gene encoding an SPI. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a gene encoding an SPI in the vector. For example, if the gene encoding the SPI is inserted within the marker gene sequence of the vector, recombinants containing the gene 35

encoding the SPI insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the gene product (i.e., SPI) expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the SPI in *in vitro* assay systems, e.g., binding with anti-SPI antibody.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered SPI or SPI-related polypeptide may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, phosphorylation of proteins). Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system will produce an unglycosylated product and expression in yeast will produce a glycosylated product. Eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, neuronal cell lines such as, for example, SK-N-AS, SK-N-FI, SK-N-DZ human neuroblastomas (Sugimoto et al, *J. Natl. Cancer Inst.* (1984) 73: 51-57), SK-N-SH human neuroblastoma (Biochim. Biophys. Acta, 1982, 704: 450-460), Daoy human cerebellar medulloblastoma (He et al, *Cancer Res.* (1992) 52: 1144-1148) DBTRG-05MG glioblastoma cells (Krause et al, *In vitro Cell. Dev. Biol.* (1992) 28A: 609-614), IMR-32 human neuroblastoma (*Cancer Res.*, (1970) 30:2110-2118), 1321N1 human astrocytoma (*Proc. Natl. Acad. Sci. USA* (1977) 74:4816), MOG-G-CGM human astrocytoma (*Br. J. Cancer*, (1984) 49:269), U87MG human glioblastoma-astrocytoma (*Acta Pathol. Microbiol. Scand.*, (1968) 74: 465-486), A172 human glioblastoma (Olrapade et al, *Cancer Res.* (1992) 52:2523- 2529), C6 rat glioma cells (Benda et al, *Science* (1968) 161:370-371), Neuro-2a mouse neuroblastoma (*Proc. Natl. Acad. Sci. USA*, (1970) 65: 129-136), NB41A3 mouse neuroblastoma (*Proc. Natl. Acad. Sci. USA*, (1962) 48:1184-1190), SCP sheep choroid plexus (Bolin et al, *J. Virol. Methods* (1994) 48: 211-221), G355-5, PG-4 Cat normal astrocyte (Hapsal et al, *J. Virol.* (1985) 53:827-833), Mpf ferret brain (Trowbridge et al, *In vitro* (1982) 18:952-960), and normal cell lines such as, for example, CTX TNA2 rat normal cortex brain (Radany et al, *Proc. Natl. Acad. Sci. USA* (1992) 89:6467-6471) such as, for example, CRL7030 and Hs578Bsr. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the differentially expressed or

pathway gene protein may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched medium, and then are switched to a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the differentially expressed or pathway gene protein. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the differentially expressed or pathway gene protein.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al, *Cell* (1977) 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szymbalska & Szymbalski, *Proc. Natl. Acad. Sci. USA* (1962) 48:2026), and adenine phosphoribosyltransferase (Lowy, et al, *Cell* (1980) 22:817) genes can be employed in tk-, hprt- or sprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al, *Proc. Natl. Acad. Sci. USA* (1980) 77:3567; O'Hare, et al, *Proc. Natl. Acad. Sci. USA* (1981) 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA* (1981) 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberro-Garcia, et al, *J. Mol. Biol.* (1981) 150:1); and hygro, which confers resistance to hygromycin (Santerre, et al, *Gene* (1984) 30:147) genes.

In other specific embodiments, the SPI, fragment, analog, or derivative may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence). For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification, increase half-life *in vivo*, and enhance the delivery of an antigen across an epithelial barrier to the immune system. An increase in the half-life *in vivo* and facilitated purification has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traumecker et al, *Nature*, (1988) 331:84-86. Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn

binding partner such as IgG or Fc fragments (see, e.g., PCT publications WO 96/22024 and WO 99/04813).

Nucleic acids encoding an SPI, a fragment of an SPI, an SPI-related polypeptide, or a fragment of an SPI-related polypeptide can be fused to an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al, allows for the ready purification of non-denatured fusion protein expressed in human cell lines (Janknecht et al, *Proc. Natl. Acad. Sci. USA* (1991) 88:8972-897).

Fusion proteins can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, a fusion protein may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

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#### 5.8 Domain Structure of SPIs

Domains of some SPIs are known in the art and have been described in the scientific literature. Moreover, domains of an SPI can be identified using techniques known to those of skill in the art. For example, one or more domains of an SPI can be identified by using one or more of the following programs: ProDom, TMpred, and SAPS. ProDom compares the amino acid sequence of a polypeptide to a database of compiled domains (see, e.g., <http://www.toulouse.inra.fr/prodom.html>; Corpet F, Gouzy J, & Kahn D, *Nucleic Acids Res.*, (1999) 27:263-267). TMpred predicts membrane-spanning regions of a polypeptide and their orientation. This program uses an algorithm that is based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins (see, e.g., [http://www.ch.cnbnet.org/software/TMPRED\\_form.html](http://www.ch.cnbnet.org/software/TMPRED_form.html); Hofmann & Stoffel, (1993) "TMbase - A database of membrane spanning proteins segments." *Biol. Chem. Hoppe-Seyler* 374,166). The SAPS program analyzes polypeptides for statistically significant features like charge-clusters, repeats, hydrophobic regions, compositional domains (see, e.g., Brendel et al, *Proc. Natl. Acad. Sci. USA* (1992) 89: 2002-2006). Thus, based on the present description, the skilled artisan can identify domains of an SPI having enzymatic or binding activity, and further can identify nucleotide sequences encoding such domains. These nucleotide sequences can then be used for recombinant expression of an SPI fragment that retains the enzymatic or binding activity of the SPI.

Based on the present description, the skilled artisan can identify domains of an SPI having enzymatic or binding activity, and further can identify nucleotide sequences encoding

such domains. These nucleotide sequences can then be used for recombinant expression of SPI fragments that retain the enzymatic or binding activity of the SPI.

In one embodiment, an SPI has an amino acid sequence sufficiently similar to an identified domain of a known polypeptide. As used herein, the term "sufficiently similar" refers to a first amino acid or nucleotide sequence which contains a sufficient number of identical or equivalent (e.g., with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have or encode a common structural domain or common functional activity or both.

An SPI domain can be assessed for its function using techniques well known to those of skill in the art. For example, a domain can be assessed for its kinase activity, or for its ability to bind to DNA using techniques known to the skilled artisan. Kinase activity can be assessed, for example, by measuring the ability of a polypeptide to phosphorylate a substrate. DNA binding activity can be assessed, for example, by measuring the ability of a polypeptide to bind to a DNA binding element in a electromobility shift assay. In a preferred embodiment, the function of a domain of an SPI is determined using an assay described in one or more of the references identified in Table XIV, *infra*.

#### 5.9 Production of Antibodies to SPIs

According to the invention an SPI, SPI analog, SPI-related protein or a fragment or derivative of any of the foregoing may be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Such immunogens can be isolated by any convenient means, including the methods described above. Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, humanized or chimeric antibodies, single chain antibodies, Fab fragments and F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (e.g., IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule.

In one embodiment, antibodies that recognize gene products of genes encoding SPIs are publicly available. For example, antibodies that recognize these SPIs and/or their isoforms include antibodies recognizing SPI-6, SPI-8, SPI-9, SPI-10, SPI-15, SPI-16, SPI-17, SPI-23, SPI-32, SPI-33, SPI-34, SPI-35, SPI-41, SPI-42, SPI-43, SPI-54, SPI-57, SPI-60, SPI-62, SPI-63, SPI-67, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-82, SPI-91, SPI-92, SPI-93, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-102, SPI-107, SPI-

- 113, SPI-114, SPI-115, SPI-122, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-143, SPI-152, SPI-154, SPI-155, SPI-164, SPI-167, SPI-170, SPI-175, SPI-183, SPI-184, SPI-185, SPI-190, SPI-193, SPI-205, SPI-211, SPI-231, SPI-233, SPI-237, SPI-242, SPI-244, SPI-246, SPI-252, SPI-254, SPI-255, SPI-258, SPI-261, SPI-264, SPI-265, SPI-269, SPI-275, SPI-285, 5 SPI-289, SPI-290, SPI-321, SPI-323, SPI-325, SPI-326, SPI-327, SPI-328, SPI-329, SPI-334, SPI-339, SPI-342, SPI-343, SPI-345, SPI-346, SPI-347, SPI-348, SPI-349, SPI-353, SPI-355, SPI-357, SPI-361, SPI-362, SPI-370, SPI-372, SPI-375, SPI-376, SPI-379, SPI-381, SPI-397, SPI-402, SPI-404, SPI-405, SPI-407, SPI-408, SPI-409, SPI-410, SPI-411, SPI-412, SPI-413, SPI-414, SPI-416, SPI-418, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, 10 SPI-427, SPI-429, SPI-431, SPI-432, SPI-433, SPI-435, SPI-438, SPI-441, SPI-443, SPI-446, SPI-448, SPI-449, SPI-452, SPI-453, SPI-454, SPI-457, SPI-461, SPI-462, SPI-464, SPI-465, SPI-467, which antibodies can be purchased from commercial sources as shown in Table VII above. In another embodiment, methods known to those skilled in the art are used to produce 15 antibodies that recognize an SPI, an SPI analog, an SPI-related polypeptide, or a derivative or fragment of any of the foregoing.

In one embodiment of the invention, antibodies to a specific domain of an SPI are produced. In a specific embodiment, hydrophilic fragments of an SPI are used as immunogens for antibody production.

- In the production of antibodies, screening for the desired antibody can be 20 accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific domain of an SPI, one may assay generated hybridomas for a product which binds to an SPI fragment containing such domain. For selection of an antibody that specifically binds a first SPI homolog but which does not specifically bind to (or binds less avidly to) a second SPI homolog, one can 25 select on the basis of positive binding to the first SPI homolog and a lack of binding to (or reduced binding to) the second SPI homolog. Similarly, for selection of an antibody that specifically binds an SPI but which does not specifically bind to (or binds less avidly to) a different isoform of the same protein (such as a different glycoform having the same core peptide as the SPI), one can select on the basis of positive binding to the SPI and a lack of 30 binding to (or reduced binding to) the different isoform (e.g., a different glycoform). Thus, the present invention provides an antibody (preferably a monoclonal antibody) that binds with greater affinity (preferably at least 2-fold, more preferably at least 5-fold still more preferably at least 10-fold greater affinity) to an SPI than to a different isoform or isoforms (e.g., glycoforms) of the SPI.

- 35 Polyclonal antibodies that may be used in the methods of the invention are heterogeneous populations of antibody molecules derived from the sera of immunized animals. Unfractionated immune serum can also be used. Various procedures known in the

- art may be used for the production of polyclonal antibodies to an SPI, a fragment of an SPI, an SPI-related polypeptide, or a fragment of an SPI-related polypeptide. In a particular embodiment, rabbit polyclonal antibodies to an epitope of an SPI or an SPI-related polypeptide can be obtained. For example, for the production of polyclonal or monoclonal antibodies, various host animals can be immunized by injection with the native or a synthetic (e.g., recombinant) version of an SPI, a fragment of an SPI, an SPI-related polypeptide, or a fragment of an SPI-related polypeptide, including but not limited to rabbits, mice, rats, etc. The Preferred Technology described herein provides isolated SPIs suitable for such immunization. If the SPI is purified by gel electrophoresis, the SPI can be used for 5 immunization with or without prior extraction from the polyacrylamide gel. Various adjuvants may be used to enhance the immunological response, depending on the host species, including, but not limited to, complete or incomplete Freund's adjuvant, a mineral gel such as aluminum hydroxide, surface active substance such as lysolecithin, pluronic polylol, a polymer, a peptide, an oil emulsion, keyhole limpet hemocyanin, dinitrophenol, and an adjuvant such as BCG (bacillus Calmette-Guerin) or Corynebacterium parvum. Additional adjuvants are also known in the art.

For preparation of monoclonal antibodies (mAbs) directed toward an SPI, a fragment of an SPI, an SPI-related polypeptide, or a fragment of an SPI-related polypeptide, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (*Nature* (1975) 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., *Immunology Today* (1983) 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., (1985) in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAbs of the invention may be cultivated *in vitro* or *in vivo*. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing known technology (PCT/US90/02545, incorporated herein by reference).

- 30 The monoclonal antibodies include but are not limited to human monoclonal antibodies and chimeric monoclonal antibodies (e.g., human-mouse chimeras). A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region derived from a murine mAb. (See, e.g., Cabilio et al., U.S. Patent No. 4,816,567; and Boss et al., U.S. 35 Patent No. 4,816,567, which are incorporated herein by reference in their entirety.) Humanized antibodies are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework

region from a human immunoglobulin molecule. (See, e.g., Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.)

Chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication 5 No. WO 87/02671; European Patent Application 184,187; European Patent Application 171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better et al., 1988, *Science* 240:1041-1043; Liu et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:3439-3443; Liu et al., *J. Immunol.* (1987) 139:3521-3526; Sun et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:214-218; 10 Nishizuka et al., *Canc. Res.* (1987) 47:999-1005; Wood et al., *Nature* (1985) 314:446-449; and Shaw et al., *J. Natl. Cancer Inst.* (1988) 80:1553-1559; Morrison, *Science* (1985) 239:1202-1207; Oi et al., *BioTechniques* (1986) 4:214; U.S. Patent 5,225,539; Jones et al., *Nature* (1986) 321:522-525; Verhoyan et al., *Science* (1988) 239:1534; and Beidler et al., *J. Immunol.* (1988) 141:4053-4060.

- 15 Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of an SPI of the invention. 20 Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing 25 human antibodies, see Lomberg and Huszar (*Int. Rev. Immunol.* (1995) 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,659,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA) and GenPharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using 30 technology similar to that described above.

35 Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *BioTechnology* (1994) 12:899-903).

- The antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles that carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labelled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* (1995) 182:41-50; Ames et al., *J. Immunol. Methods* (1995) 184:177-186; Kentleborough et al., *Eur. J. Immunol.* (1994) 24:952-958; Persic et al., *Gene* (1997) 187:9-18; Burton et al., *Advances in Immunology* (1994) 57:191-280 ; PCT Application No. PCT/GB91/01134; PCT Publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO '95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,725; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

- 20 As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies; or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mulligan et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., (1995) AJRI 34:26-34 ; and Better et al., *Science* (1983) 240:1041-1043 (said references incorporated by reference in their entireties).

- 25 Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Sun et al., *Proc. Natl. Acad. Sci. USA* (1993) 90:7995-7999; and Stern et al., *Science* (1985) 240:1038-1040.

- 30 The invention further provides for the use of bispecific antibodies, which can be made by methods known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Milstein et al., *Nature* (1983) 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas

(quadrernas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Trampecker et al., *EMBO J.* (1991) 10:3655-3659.

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant region, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published March 3, 1994. For further details for generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology* (1986) 121:210.

The invention provides functionally active fragments, derivatives or analogs of the anti-SPI immunoglobulin molecules. Functionally active means that the fragment, derivative or analog is able to elicit anti-anti-idiotypic antibodies (i.e., tertiary antibodies) that recognize the same antigen that is recognized by the antibody from which the fragment, derivative or analog is derived. Specifically, in a preferred embodiment the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR

sequences can be used in binding assays with the antigens by any binding assay method known in the art.

The present invention provides antibody fragments such as, but not limited to, F(ab')2 fragments and Fab fragments. Antibody fragments which recognize specific epitopes may be generated by known techniques. F(ab')2 fragments consist of the variable region, the light chain constant region and the CH1 domain of the heavy chain and are generated by pepsin digestion of the antibody molecule. Fab fragments are generated by reducing the disulfide bridges of F(ab')2 fragments. The invention also provides heavy chain and light chain dimers of the antibodies of the invention, or any minimal fragment thereof such as Fv or single chain antibodies (SCAs) (e.g., as described in U.S. Patent 4,946,778; Bird, *Science* (1988) 242:423-42; Huston et al., *Proc. Natl. Acad. Sci. USA* (1988) 85:5879-5883; and Ward et al., *Nature* (1989) 334:544-54), or any other molecule with the same specificity as the antibody of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may be used (Skerra et al., *Science* (1988) 242:1038-1041).

In other embodiments, the invention provides fusion proteins of the immunoglobulins of the invention (or functionally active fragments thereof), for example in which the immunoglobulin is fused via a covalent bond (e.g., a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the immunoglobulin. Preferably the immunoglobulin, or fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. As stated above, such fusion proteins may facilitate purification, increase half-life *in vivo*, and enhance the delivery of an antigen across an epithelial barrier to the immune system.

The immunoglobulins of the invention include analogs and derivatives that are either modified, i.e., by the covalent attachment of any type of molecule as long as such covalent attachment that does not impair immunospecific binding. For example, but not by way of limitation, the derivatives and analogs of the immunoglobulins include those that have been further modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the analog or derivative may contain one or more non-classical amino acids.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the SPIs of the invention, e.g., for imaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

#### 5.10 Expression Of Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

Recombinant expression of antibodies, or fragments, derivatives or analogs thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutzmeier et al., *BioTechniques* (1994) 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, the nucleic acid encoding the antibody may be obtained by cloning the antibody. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody may be obtained from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody molecule that specifically recognizes a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an antibody), antibodies specific for a particular antigen may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies. Alternatively, a clone encoding at least the Fab portion of the antibody may be obtained by screening Fab expression libraries (e.g., as described in Huse et al., *Science* (1989) 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clarkson et al., *Nature* (1991) 352:624; Hane et al., *Proc. Natl. Acad. Sci. USA* (1997) 94:4937).

Once a nucleic acid encoding at least the variable domain of the antibody molecule is obtained, it may be introduced into a vector containing the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a

complete antibody molecule are also available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitution(s) or deletion(s) necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulhydryl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical mutagenesis, *in vitro* site directed mutagenesis (Hutchinson et al., *J. Biol. Chem.* (1978) 253:6551), PCT based methods, etc.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci.* (1984) 81:851-855; Neuberger et al., *Nature* (1984) 312:604-608; Takeda et al., *Nature* (1985) 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human antibody constant region, e.g., humanized antibodies.

Once a nucleic acid encoding an antibody molecule of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the protein of the invention by expressing nucleic acid containing the antibody molecule sequences are described hereinafter. Methods which are well known to those skilled in the art can be used to construct expression vectors containing an antibody molecule coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook et al., (1990, Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Ausubel et al. (eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY).

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention.

The host cells used to express a recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli*, or, preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective

expression system for antibodies (Foecking et al, *Gene* (1986) 45:101; Cockett et al, *Bio/Technology* (1990) 8:2).

A variety of host-expression vector systems may be utilized to express an antibody molecule of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the antibody molecule of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions comprising an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al, *EMBO J.* (1983) 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* (1985) 13:3101-3109; Van Heege & Schuster, *J. Biol. Chem.* (1989) 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for

example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). In mammalian host cells, a number of viral-based expression systems (e.g., an adenovirus expression system) may be utilized.

As discussed above, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein.

For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cell lines that stably express an antibody of interest can be produced by transfecting the cells with an expression vector comprising the nucleotide sequence of the antibody and the nucleotide sequence of a selectable (e.g., neomycin or hygromycin), and selecting for expression of the selectable marker. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

The expression level of the antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3, (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al, 1983, *Mol. Cell. Biol.* 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature* (1986) 322:52; Kohler, *Proc. Natl. Acad. Sci. USA* (1980) 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once the antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an antibody molecule, for example, by chromatography (e.g., ion exchange chromatography, affinity chromatography such as with protein A or specific antigen, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al, allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al, *Proc. Natl. Acad. Sci. USA* (1991) 88:8972-897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni<sup>2+</sup>-nitrotriacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

#### 5.11 Conjugated Antibodies

In a preferred embodiment, anti-SPI antibodies or fragments thereof are conjugated to a diagnostic or therapeutic moiety. The antibodies can be used for diagnosis or to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbellifluorone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlororubiazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luciferin; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include 125I, 131I, 111In and 99Tc.

Anti-SPI antibodies or fragments thereof can be conjugated to a therapeutic agent or drug moiety to modify a given biological response. The therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6),

granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Amon et al, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reinfeld et al, (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al, (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al, (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabelled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al, (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

An antibody with or without a therapeutic moiety conjugated to it can be used as a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

#### 5.12 Diagnosis of Schizophrenia

In accordance with the present invention, test samples of cerebrospinal fluid (CSF), serum, plasma or urine obtained from a subject suspected of having or known to have Schizophrenia can be used for diagnosis or monitoring. In one embodiment, a decreased abundance of one or more SFs or SPIs (or any combination of them) in a test sample relative to a control sample (from a subject or subjects free from Schizophrenia) or a previously determined reference range indicates the presence of Schizophrenia; SFs and SPIs suitable for this purpose are identified in Tables I and IV, respectively, as described in detail above. In another embodiment of the invention, an increased abundance of one or more SFs or SPIs (or any combination of them) in a test sample compared to a control sample or a previously determined reference range indicates the presence of Schizophrenia; SFs and SPIs suitable for this purpose are identified in Tables II and V, respectively, as described in detail above. In another embodiment, the relative abundance of one or more SFs or SPIs (or any combination of them) in a test sample compared to a control sample or a previously determined reference range indicates a subtype of Schizophrenia (e.g., familial or sporadic Schizophrenia). In yet another embodiment, the relative abundance of one or more SFs or SPIs (or any combination of them) in a test sample relative to a control sample or a previously determined reference

range indicates the degree or severity of Schizophrenia. In any of the aforesaid methods, detection of one or more SPIs described herein may optionally be combined with detection of one or more additional biomarkers for Schizophrenia including, but not limited to. Any suitable method in the art can be employed to measure the level of SPIs and SPIs, including but not limited to the Preferred Technology described herein, kinase assays, immunoassays to detect and/or visualize the SPI (e.g., Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.). In cases where an SPI has a known function, an assay for that function may be used to measure SPI expression. In a further embodiment, a decreased abundance of mRNA encoding one or more SPIs identified in Table IV (or any combination of them) in a test sample relative to a control sample or a previously determined reference range indicates the presence of Schizophrenia. In yet a further embodiment, an increased abundance of mRNA encoding one or more SPIs identified in Table V (or any combination of them) in a test sample relative to a control sample or previously determined reference range indicates the presence of Schizophrenia.

Any suitable hybridization assay can be used to detect SPI expression by detecting and/or visualizing mRNA encoding the SPI (e.g., Northern assays, dot blots, in situ hybridization, etc.).

In another embodiment of the invention, labelled antibodies, derivatives and analogs thereof, which specifically bind to an SPI can be used for diagnostic purposes to detect, diagnose, or monitor Schizophrenia. Preferably, Schizophrenia is detected in an animal, more preferably in a mammal and most preferably in a human.

### 5.13 Screening Assays

The invention provides methods for identifying agents (e.g., candidate compounds or test compounds) that bind to an SPI or have a stimulatory or inhibitory effect on the expression or activity of an SPI. The invention also provides methods of identifying agents, candidate compounds or test compounds that bind to an SPI-related polypeptide or an SPI fusion protein or have a stimulatory or inhibitory effect on the expression or activity of an SPI-related polypeptide or an SPI fusion protein. Examples of agents, candidate compounds or test compounds include, but are not limited to, nucleic acids (e.g., DNA and RNA), carbohydrates, lipids, proteins, peptides, peptidomimetics, small molecules and other drugs. Agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide, oligomer or small

molecule libraries of compounds (Lam, *Anticancer Drug Des.* (1997) 12:145; U.S. Patent No. 5,738,996; and U.S. Patent No. 5,807,683, each of which is incorporated herein in its entirety by reference).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al, *Proc. Natl. Acad. Sci. USA* (1993) 90:6909; Erb et al, *Proc. Natl. Acad. Sci. USA* (1994) 91:1422; Zuckermann et al, *J. Med. Chem.* (1994) 37:2678; Cho et al, *Science* (1993) 261:1303; Carroll et al, *Angew. Chem. Int. Ed. Engl.* (1994) 33:2059; Carroll et al, *Angew. Chem. Int. Ed. Engl.* (1994) 33:2061; and Gallop et al, *J. Med. Chem.* (1994) 37:1233, each of which is incorporated herein in its entirety by reference.

Libraries of compounds may be presented, e.g., presented in solution (e.g., Houghten, *BioTechniques* (1992) 13:412-421), or on beads (Lam, *Nature* (1991) 354:82-84), chips (Fodor, *Nature* (1993) 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (Patent Nos. 5,571,693; 5,403,484; and 5,223,409), plasmids (Cull et al, *Proc. Natl. Acad. Sci. USA* (1992) 89:1865-1869) or phage (Scott and Smith, *Science* (1990) 249:386-390; Devlin, *Science* (1990) 249:404-406; Cwirka et al, *Proc. Natl. Acad. Sci. USA* (1990) 87:6378-6382; and Felici, *J. Mol. Biol.* (1990) 222:301-310), each of which is incorporated herein in its entirety by reference.

In one embodiment, agents that interact with (i.e., bind to) an SPI, an SPI fragment (e.g., a functionally active fragment), an SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein are identified in a cell-based assay system. In accordance with this embodiment, cells expressing an SPI, a fragment of an SPI, an SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein are contacted with a candidate compound or a control compound and the ability of the candidate compound to interact with the SPI is determined. If desired, this assay may be used to screen a plurality (e.g., a library) of candidate compounds. The cell, for example, can be of prokaryotic origin (e.g., E. coli) or eukaryotic origin (e.g., yeast or mammalian). Further, the cells can express the SPI, fragment of the SPI, SPI-related polypeptide, a fragment of the SPI-related polypeptide, or an SPI fusion protein endogenously or be genetically engineered to express the SPI, fragment of the SPI, SPI-related polypeptide, a fragment of the SPI-related polypeptide, or an SPI fusion protein. In certain instances, the SPI, fragment of the SPI, SPI-related polypeptide, a fragment of the SPI-related polypeptide, or an SPI fusion protein or the candidate compound is labelled, for example with a radioactive label (such as  $^{32}P$ ,  $^{35}S$  or  $^{125}I$ ) or a fluorescent label (such as fluorescein isothiocyanate, rhodamine, phycoerythrin, phycoerythrin, allophycocyanin, o-phthaldehyde or fluorescamine) to enable detection of an interaction between an SPI and a candidate compound. The ability of the candidate compound to interact directly or indirectly with an SPI, a fragment of an SPI, an SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein can be

determined by methods known to those of skill in the art. For example, the interaction between a candidate compound and an SPI, a fragment of an SPI, an SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein can be determined by flow cytometry, a scintillation assay, immunoprecipitation or western blot analysis.

In another embodiment, agents that interact with (i.e., bind to) an SPI, an SPI fragment (e.g., a functionally active fragment) an SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein are identified in a cell-free assay system. In accordance with this embodiment, a native or recombinant SPI or fragment thereof, or a native or recombinant SPI-related polypeptide or fragment thereof, or an SPI-fusion protein or fragment thereof, is contacted with a candidate compound or a control compound and the ability of the candidate compound to interact with the SPI or SPI-related polypeptide, or SPI fusion protein is determined. If desired, this assay may be used to screen a plurality (e.g., a library) of candidate compounds. Preferably, the SPI, SPI fragment, SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI-fusion protein is first immobilized, by, for example, contacting the SPI, SPI fragment, SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein with an immobilized antibody which specifically recognizes and binds it, or by contacting a purified preparation of the SPI, SPI fragment, SPI-related polypeptide, fragment of an SPI-related polypeptide, or an SPI fusion protein with a surface designed to bind proteins. The SPI, SPI fragment, SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein may be partially or completely purified (e.g., partially or completely free of other polypeptides) or part of a cell lysate. Further, the SPI, SPI fragment, SPI-related polypeptide, a fragment of an SPI-related polypeptide may be a fusion protein comprising the SPI or a biologically active portion thereof, or SPI-related polypeptide and a domain such as glutathione-S-transferase. Alternatively, the SPI, SPI fragment, SPI-related polypeptide, fragment of an SPI-related polypeptide or SPI fusion protein can be biotinylated using techniques well known to those of skill in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, IL). The ability of the candidate compound to interact with an SPI, SPI fragment, SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein can be determined by methods known to those of skill in the art.

In another embodiment, a cell-based assay system is used to identify agents that bind to or modulate the activity of a protein, such as an enzyme, or a biologically active portion thereof, which is responsible for the production or degradation of an SPI or is responsible for the post-translational modification of an SPI. In a primary screen, a plurality (e.g., a library) of compounds are contacted with cells that naturally or recombinantly express (i) an SPI, an isoform of an SPI, an SPI homolog or SPI-related polypeptide, an SPI fusion protein, or a biologically active fragment of any of the foregoing; and (ii) a protein that is responsible for

processing of the SPI, SPI isoform, SPI homolog, SPI-related polypeptide, SPI fusion protein, or fragment in order to identify compounds that modulate the production, degradation, or post-translational modification of the SPI, SPI isoform, SPI homolog, SPI-related polypeptide, SPI fusion protein or fragment. If desired, compounds identified in the primary screen can then be assayed in a secondary screen against cells naturally or recombinantly expressing the specific SPI of interest. The ability of the candidate compound to modulate the production, degradation or post-translational modification of an SPI, isoform, homolog, SPI-related polypeptide, or SPI fusion protein can be determined by methods known to those of skill in the art, including without limitation, flow cytometry, a scintillation assay, immunoprecipitation and western blot analysis.

In another embodiment, agents that competitively interact with (i.e., bind to) an SPI, SPI fragment, SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein are identified in a competitive binding assay. In accordance with this embodiment, cells expressing an SPI, SPI fragment, SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein are contacted with a candidate compound and a compound known to interact with the SPI, SPI fragment, SPI-related polypeptide, a fragment of an SPI-related polypeptide or an SPI fusion protein; the ability of the candidate compound to competitively interact with the SPI, SPI fragment, SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein is then determined. Alternatively, agents that competitively interact with (i.e., bind to) an SPI, SPI fragment, SPI-related polypeptide or fragment of an SPI-related polypeptide are identified in a cell-free assay system by contacting an SPI, SPI fragment, SPI-related polypeptide, fragment of an SPI-related polypeptide, or an SPI fusion protein with a candidate compound and a compound known to interact with the SPI, SPI-related polypeptide or SPI fusion protein. As stated above, the ability of the candidate compound to interact with an SPI, SPI fragment, SPI-related polypeptide, a fragment of an SPI-related polypeptide, or an SPI fusion protein can be determined by methods known to those of skill in the art. These assays, whether cell-based or cell-free, can be used to screen a plurality (e.g., a library) of candidate compounds.

In another embodiment, agents that modulate (i.e., upregulate or downregulate) the expression of an SPI, or an SPI-related polypeptide are identified by contacting cells (e.g., cells of prokaryotic origin or eukaryotic origin) expressing the SPI, or SPI-related polypeptide with a candidate compound or a control compound (e.g., phosphate buffered saline (PBS)) and determining the expression of the SPI, SPI-related polypeptide, or SPI fusion protein, mRNA encoding the SPI, or mRNA encoding the SPI-related polypeptide. The level of expression of a selected SPI, SPI-related polypeptide, mRNA encoding the SPI, or mRNA encoding the SPI-related polypeptide in the presence of the candidate compound is compared to the level of expression of the SPI, SPI-related polypeptide, mRNA encoding the

SPI, or mRNA encoding the SPI-related polypeptide in the absence of the candidate compound (e.g., in the presence of a control compound). The candidate compound can then be identified as a modulator of the expression of the SPI, or an SPI-related polypeptide based on this comparison. For example, when expression of the SPI or mRNA is significantly greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of expression of the SPI or mRNA. Alternatively, when expression of the SPI or mRNA is significantly less in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of the expression of the SPI or mRNA. The level of expression of an SPI or the mRNA that encodes it can be determined by methods known to those of skill in the art. For example, mRNA expression can be assessed by Northern blot analysis or RT-PCR, and protein levels can be assessed by western blot analysis.

In another embodiment, agents that modulate the activity of an SPI, or an SPI-related polypeptide are identified by contacting a preparation containing the SPI or SPI-related polypeptide, or cells (e.g., prokaryotic or eukaryotic cells) expressing the SPI or SPI-related polypeptide with a test compound or a control compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the SPI or SPI-related polypeptide. The activity of an SPI or an SPI-related polypeptide can be assessed by detecting induction of a cellular signal transduction pathway of the SPI or SPI-related polypeptide (e.g., intracellular Ca<sup>2+</sup>, diacylglycerol, IP<sub>3</sub>, etc.), detecting catalytic or enzymatic activity of the target on a suitable substrate, detecting the induction of a reporter gene (e.g., a regulatory element that is responsive to an SPI or an SPI-related polypeptide and is operably linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a cellular response, for example, cellular differentiation, or cell proliferation. Based on the present description, techniques known to those of skill in the art can be used for measuring these activities (see, e.g., U.S. Patent No. 5,401,639, which is incorporated herein by reference). The candidate compound can then be identified as a modulator of the activity of an SPI or SPI-related polypeptide by comparing the effects of the candidate compound to the control compound. Suitable control compounds include phosphate buffered saline (PBS) and normal saline (NS).

In another embodiment, agents that modulate (i.e., upregulate or downregulate) the expression, activity or both the expression and activity of an SPI or SPI-related polypeptide are identified in an animal model. Examples of suitable animals include, but are not limited to, mice, rats, rabbits, monkeys, guinea pigs, dogs and cats. Preferably, the animal used represent a model of Schizophrenia (e.g., Phencyclidine treated rodents (Sams-Dodd *Rev Neurosci* (1999) 10, 59-90), an animal model of deficient sensorimotor gating (Swerdlow and Geyer *Schizophr Bull* (1998) 24:2 285-301), neonatal insult to the hippocampal region

(Beauregard and Bachevier *Can J Psychiatry* (1996) Sep 41:7 446-56), models based on neonatal excitotoxic hippocampal damage (Lillrank et al., *Clin Neurosci* (1995) 3:2 98-104), attention deficit models (Feldon et al., *J Psychiatr Res* 4, 345-66) and NMDA deficient rodent models (Mohn et al., *Cell* (1999) 98, 427-436). In accordance with this embodiment, the test compound or a control compound is administered (e.g., orally, rectally or parenterally such as intraperitoneally or intravenously) to a suitable animal and the effect on the expression, activity or both expression and activity of the SPI or SPI-related polypeptide is determined. Changes in the expression of an SPI or SPI-related polypeptide can be assessed by the method outlined above.

In yet another embodiment, an SPI or SPI-related polypeptide is used as a "bait protein" in a two-hybrid assay or three hybrid assay to identify other proteins that bind to or interact with an SPI or SPI-related polypeptide (see, e.g., U.S. Patent No. 5,283,317; Zervos et al., *Cell* (1993) 72:223-232; Madura et al., *J. Biol. Chem.* (1993) 268:12046-12054; Bartel et al., *BioTechniques* (1993) 14:920-924; Iwabuchi et al., *Oncogene* (1993) 8:1693-1696; and PCT Publication No. WO 94/10300). As those skilled in the art will appreciate, such binding proteins are also likely to be involved in the propagation of signals by the SPIs of the invention as, for example, upstream or downstream elements of a signaling pathway involving the SPIs of the invention.

Table XIV enumerates scientific publications describing suitable assays for detecting or quantifying enzymatic or binding activity of an SPI, an SPI analog, an SPI-related polypeptide, or a fragment of any of the foregoing. Each such reference is hereby incorporated in its entirety. In a preferred embodiment, as assay referenced in Table XIV is used in the screens and assays described herein, for example to screen for or identify a compound that modulates the activity of (or that modulates both the expression and activity of) an SPI, SPI analog, or SPI-related polypeptide, a fragment of any of the foregoing.

Table XIV.

SPI	References
SPI-82	Structural Biology 2000 7: 312-321,
SPI-109	J. Am. Chem. Soc. 2000 122: 2178-2192,
SPI-154	
SPI-188	
SPI-3	Clin Chem 1993 Feb 39(2): 309-12
SPI-32	J Immunol Methods 1987 Aug 24 102:1 7-14
SPI-33	
SPI-45	
SPI-92	
SPI-122	
SPI-165	

SPI-57	J Clin Lab Immunol 1986 Dec 21(4): 201-7
SPI-67	
SPI-74	
SPI-77	
SPI-107	
SPI-153	
SPI-162	
SPI-164	
SPI-175	
SPI-186	
SPI-205	
SPI-216	
SPI-41	Neuroendocrinology 1992 Mar 55:3 308-16
SPI-47	J Chromatogr 1987 Dec 18 411: 498-501 Eisci Shikken Hoikoku 1972 90: 89-92 Analyst 1990 Aug 115:8 1143-4
SPI-194	Biochem J 1997 Mar 1 322 ( Pt 2): 455-60; Biochem Soc Trans 1997 Nov 25:4 S591; Biochim Biophys Acta 1986 Oct 10 888:3 325-31 <a href="http://www.promega.com">http://www.promega.com</a>

This invention further provides novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

#### 5.14 Therapeutic Uses of SPIs

The invention provides for treatment or prevention of various diseases and disorders by administration of a therapeutic compound. Such compounds include but are not limited to: SPIs, SPI analogs, SPI-related polypeptides and derivatives (including fragments) thereof; antibodies to the foregoing nucleic acids encoding SPIs, SPI analogs, SPI-related polypeptides and fragments thereof; antisense nucleic acids to a gene encoding an SPI or SPI-related polypeptide; and modulators (e.g., agonists and antagonists) of a gene encoding an SPI or SPI-related polypeptide. An important feature of the present invention is the identification of genes encoding SPIs involved in Schizophrenia. Schizophrenia can be treated (e.g., to ameliorate symptoms or to retard onset or progression) or prevented by administration of a therapeutic compound that promotes function or expression of one or more SPIs that are decreased in the CSF of Schizophrenia subjects having Schizophrenia, or by administration of a therapeutic compound that reduces function or expression of one or more SPIs that are increased in the CSF of subjects having Schizophrenia.

In one embodiment, one or more antibodies each specifically binding to an SPI are administered alone or in combination with one or more additional therapeutic compounds or treatments. Examples of such therapeutic compounds or treatments include, but are not limited to, Scirindole, Haloperidol, Pirenzepine, Perazine, Risperdal, Farnotidine, Clozarell,

Mesoridazine, Quetiapine, atypical anti-psychotic medications of Risperidone, Zyprexa (Olanzapine) and Clozapine and any other Dibenzothiazepines. The compounds of the invention may be given in combination with any other compound, including Scirindole, Haloperidol, Pirenzepine, Perazine, Risperdal, Farnotidine, Clozarell, Mesoridazine, Quetiapine, atypical anti-psychotic medications of Risperidone, Zyprexa (Olanzapine) and Clozapine and any other Dibenzothiazepines.

Preferably, a biological product such as an antibody is allogeneic to the subject to which it is administered. In a preferred embodiment, a human SPI or a human SPI-related polypeptide, a nucleotide sequence encoding a human SPI or a human SPI-related polypeptide, or an antibody to a human SPI or a human SPI-related polypeptide, is administered to a human subject for therapy (e.g., to ameliorate symptoms or to retard onset or progression) or prophylaxis.

#### 5.14.1 Treatment And Prevention Of Schizophrenia

Schizophrenia is treated or prevented by administration to a subject suspected of having or known to have Schizophrenia or to be at risk of developing Schizophrenia of a compound that modulates (i.e., increases or decreases) the level or activity (i.e., function) of one or more SPIs or the level of one or more SFs that are differentially present in the CSF of subjects having Schizophrenia compared with CSF of subjects free from Schizophrenia. In one embodiment, Schizophrenia is treated or prevented by administering to a subject suspected of having or known to have Schizophrenia or to be at risk of developing Schizophrenia a compound that upregulates (i.e., increases) the level or activity (i.e., function) of one or more SPIs or the level of one or more SFs that are decreased in the CSF of subjects having Schizophrenia. In another embodiment, a compound is administered that downregulates the level or activity (i.e., function) of one or more SPIs or the level of one or more SFs that are increased in the CSF of subjects having Schizophrenia. Examples of such a compound include but are not limited to: SPIs, SPI fragments and SPI-related polypeptides; nucleic acids encoding an SPI, an SPI fragment and an SPI-related polypeptide (e.g., for use in gene therapy); and, for those SPI or SPI-related polypeptides with enzymatic activity, compounds or molecules known to modulate that enzymatic activity. Other compounds that can be used, e.g., SPI agonists, can be identified using *in vitro* assays.

Schizophrenia is also treated or prevented by administration to a subject suspected of having or known to have Schizophrenia or to be at risk of developing Schizophrenia of a compound that downregulates the level or activity of one or more SPIs or the level of one or more SFs that are increased in the CSF of subjects having Schizophrenia. In another embodiment, a compound is administered that upregulates the level or activity of one or more SPIs or the level of one or more SFs that are decreased in the CSF of subjects having Schizophrenia.

Schizophrenia. Examples of such a compound include, but are not limited to, SPI antisense oligonucleotides, ribozymes, antibodies directed against SPIs, and compounds that inhibit the enzymatic activity of an SPI. Other useful compounds e.g., SPI antagonists and small molecule SPI antagonists, can be identified using *in vitro* assays.

In a preferred embodiment, therapy or prophylaxis is tailored to the needs of an individual subject. Thus, in specific embodiments, compounds that promote the level or function of one or more SPIs, or the level of one or more SFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have Schizophrenia, in whom the levels or functions of said one or more SPIs, or levels of said one or more SFs, are absent or are decreased relative to a control or normal reference range. In further embodiments, compounds that promote the level or function of one or more SPIs, or the level of one or more SFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have Schizophrenia in whom the levels or functions of said one or more SPIs, or levels of said one or more SFs, are increased relative to a control or to a reference range. In further embodiments, compounds that decrease the level or function of one or more SPIs, or the level of one or more SFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have Schizophrenia in whom the levels or functions of said one or more SPIs, or levels of said one or more SFs, are increased relative to a control or to a reference range. In further embodiments, compounds that decrease the level or function of one or more SPIs, or the level of one or more SFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have Schizophrenia in whom the levels or functions of said one or more SPIs, or levels of said one or more SFs, are decreased relative to a control or to a reference range. The change in SPI function or level, or SF level, due to the administration of such compounds can be readily detected, e.g., by obtaining a sample (e.g., a sample of CSF, blood or urine or a tissue sample such as biopsy tissue) and assaying *in vitro* the levels of said SPIs or the levels or activities of said SFs, or the levels of mRNAs encoding said SPIs, or any combination of the foregoing. Such assays can be performed before and after the administration of the compound as described herein.

The compounds of the invention include but are not limited to any compound, e.g., a small organic molecule, protein, peptide, antibody, nucleic acid, etc. that restores the Schizophrenia SPI or SF profile towards normal with the proviso that such compounds do not include Haloperidol, Pimozide, Perazine, Risperidol, Famotidine, Clozaril, Mesoridazine, Quetiapine, atypical anti-psychotic medications of Risperidone, Zyprexa (Olanzapine) and Clozapine and any other Dibenzothiazepines.

#### 5.14.2 Gene Therapy

Bioistic, Dupont); by coating with lipids, cell-surface receptors or transfecting agents; by encapsulation in liposomes, microparticles or microcapsules; by administering it in linkage to a peptide which is known to enter the nucleus; or by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, *J. Biol. Chem.* (1987) 262:4429-4432), which can be used to target cell types specifically expressing the receptors. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180 dated April 16, 1992 (Wu et al.); WO 92/22635 dated December 23, 1992 (Wilson et al.); WO 92/20316 dated November 26, 1992 (Findics et al.); WO 93/14188 dated July 22, 1993 (Clarke et al.), WO 93/20221 dated October 14, 1993 (Young)). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., *Nature* (1989) 342:435-438).

In a specific embodiment, a viral vector that contains a nucleic acid encoding an SPI is used. For example, a retroviral vector can be used (see Miller et al., *Meth. Enzymol.* (1993) 217:581-599). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The nucleic acid encoding the SPI to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a subject. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* (1994) 6:291-302, which describes the use of a retroviral vector to deliver the mdrl gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy art: Clowes et al., *J. Clin. Invest.* (1994) 93:644-651; Kian et al., *Blood* (1994) 83:1467-1473; Salmons and Gunzberg, *Human Gene Therapy* (1993) 4:129-141; and Grossman and Wilson, *Curr. Opin. in Genetics and Devol.* (1993) 3:110-114.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Koizaki and Wilson, *Current Opinion in Genetics and Development* (1993) 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* (1994) 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* (1991) 252:431-434; Rosenfeld et al., *Cell* (1992) 68:143-

In a specific embodiment, nucleic acids comprising a sequence encoding an SPI, an SPI fragment, SPI-related polypeptide or fragment of an SPI-related polypeptide, are administered to promote SPI function by way of gene therapy. Gene therapy refers to administration to a subject of an expressed or expressible nucleic acid. In this embodiment, the nucleic acid produces its encoded polypeptide that mediates a therapeutic effect by promoting SPI function.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* (1993) 12:488-505; Wu and Wu, *Biotherapy* (1991) 3:87-95; Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* (1993) 32:573-596; Mulligan, *Science* (1993) 260:926-932; and Morgan and Anderson, *Ann. Rev. Biochem.* (1993) 62:191-217; May, 1993, *TIBTECH* 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al., (eds.), 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; and Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY.

In a preferred aspect, the compound comprises a nucleic acid encoding an SPI or fragment or chimeric protein thereof, said nucleic acid being part of an expression vector that expresses an SPI or fragment or chimeric protein thereof in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the SPI coding region, said promoter being inducible or constitutive (and, optionally, tissue-specific). In another particular embodiment, a nucleic acid molecule is used in which the SPI coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the SPI nucleic acid (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* (1989) 86:8932-8935; Zijlstra et al., *Nature* (1989) 342:435-438).

Delivery of the nucleic acid into a subject may be direct, in which case the subject is directly exposed to the nucleic acid or nucleic acid-carrying vector; this approach is known as *in vivo* gene therapy. Alternatively, delivery of the nucleic acid into the subject may be indirect, in which case cells are first transformed with the nucleic acid *in vitro* and then transplanted into the subject; this approach is known as *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286); by direct injection of naked DNA; by use of microparticle bombardment (e.g., a gene gun;

155; Mastrangeli et al., *J. Clin. Invest.* (1993) 91:225-234; PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* (1995) 2:775-783.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* (1993) 204:289-300; U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* (1993) 217:599-618; Cohen et al., *Meth. Enzymol.* (1993) 217:618-644; Cline, *Pharmac. Ther.* (1985) 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a subject by various methods known in the art. In a preferred embodiment, epithelial cells are injected, e.g., subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the subject. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, the condition of the subject, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to neuronal cells, glial cells (e.g., oligodendrocytes or astrocytes), epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood or fetal liver.

In a preferred embodiment, the cell used for gene therapy is autologous to the subject that is treated.

In an embodiment in which recombinant cells are used in gene therapy, a nucleic acid encoding an SPI is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem or progenitor cells which can be isolated and maintained *in vitro* can be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598, dated April 28, 1994; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, *Meth. Cell Bio.* (1980) 21A:229; and Pitalkow and Scott, *Mayo Clinic Proc.* (1986) 61:771).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Direct injection of a DNA coding for an SPI may also be performed according to, for example, the techniques described in United States Patent No. 5,589,466. These techniques involve the injection of "naked DNA", i.e., isolated DNA molecules in the absence of liposomes, cells, or any other material besides a suitable carrier. The injection of DNA encoding a protein and operably linked to a suitable promoter results in the production of the protein in cells near the site of injection and the elicitation of an immune response in the subject to the protein encoded by the injected DNA. In a preferred embodiment, naked DNA comprising (a) DNA encoding an SPI and (b) a promoter are injected into a subject to elicit an immune response to the SPI.

#### 5.14.3 Inhibition of SPIs to Treat Schizophrenia

In one embodiment of the invention, Schizophrenia is treated or prevented by administration of a compound that antagonizes (inhibits) the level(s) and/or function(s) of one or more SPIs which are elevated in the CSF of subjects having Schizophrenia as compared with CSF of subjects free from Schizophrenia. Compounds useful for this purpose include but are not limited to anti-SPI antibodies (and fragments and derivatives containing the binding region thereof), SPI antisense or ribozyme nucleic acids, and nucleic acids encoding dysfunctional SPIs that are used to "knockout" endogenous SPI function by homologous recombination (see, e.g., Capocchi, *Science* (1989) 244:1288-1292). Other compounds that inhibit SPI function can be identified by use of known *in vitro* assays, e.g., assays for the ability of a test compound to inhibit binding of an SPI to another protein or a binding partner, or to inhibit a known SPI function. Preferably such inhibition is assayed *in vitro* or in cell culture, but genetic assays may also be employed. The Preferred Technology can also be

used to detect levels of the SPI before and after the administration of the compound. Preferably, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific compound and whether its administration is indicated for treatment of the affected tissue, as described in more detail below.

5 In a specific embodiment, a compound that inhibits an SPI function is administered therapeutically or prophylactically to a subject in whom an increased CSF level or functional activity of the SPI (e.g., greater than the normal level or desired level) is detected as compared with CSF of subjects free from Schizophrenia or a predetermined reference range. Methods standard in the art can be employed to measure the increase in an SPI level or 10 function, as outlined above. Preferred SPI inhibitor compositions include small molecules, i.e., molecules of 1000 daltons or less. Such small molecules can be identified by the screening methods described herein.

#### 5.14.4 Antisense Regulation of SPIs

15 In a specific embodiment, SPI expression is inhibited by use of SPI antisense nucleic acids. The present invention provides the therapeutic or prophylactic use of nucleic acids comprising at least six nucleotides that are antisense to a gene or cDNA encoding an SPI or a portion thereof. As used herein, an SPI "antisense" nucleic acid refers to a nucleic acid capable of hybridizing by virtue of some sequence complementarity to a portion of an RNA (preferably mRNA) encoding an SPI. The antisense nucleic acid may be complementary to a coding and/or noncoding region of an mRNA encoding an SPI. Such antisense nucleic acids have utility as compounds that inhibit SPI expression, and can be used in the treatment or prevention of Schizophrenia.

The antisense nucleic acids of the invention are double-stranded or single-stranded oligonucleotides, RNA or DNA or a modification or derivative thereof, and can be directly administered to a cell or produced intracellularly by transcription of exogenous, introduced sequences.

20 The invention further provides pharmaceutical compositions comprising an effective amount of the SPI antisense nucleic acids of the invention in a pharmaceutically acceptable carrier, as described *infra*.

25 In another embodiment, the invention provides methods for inhibiting the expression of an SPI nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an SPI antisense nucleic acid of the invention.

30 SPI antisense nucleic acids and their uses are described in detail below.

#### 5.14.5 SPI Antisense Nucleic Acids

The SPI antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides ranging from 6 to about 50 oligonucleotides. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof and can be single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appended groups such as peptide; agents that facilitate transport across the cell membrane (see, e.g., Lettinger et al, *Proc. Natl. Acad. Sci. USA* (1989) 86:6553-6556; Lemaitre et al, *Proc. Natl. Acad. Sci. USA* (1987) 84:648-652; PCT Publication No. WO 88/09810, published December 15, 1988) or blood-brain barrier (see, e.g., PCT Publication No. WO 89/10134, published April 25, 1988); hybridization-triggered cleavage agents (see, e.g., Krosl et al, *BioTechniques* (1988) 6:958-976) or intercalating agents (see, e.g., Zan, *Pharm. Res.* (1988) 5:539-549).

In a preferred aspect of the invention, an SPI antisense oligonucleotide is provided, preferably of single-stranded DNA. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

The SPI antisense oligonucleotide may comprise at least one of the following modified base moieties: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, queosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxymethyl-2-thiouracil, beta-D-ribofuranose, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp)w, 2,6-diaminopurine, and other base analogs.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety, e.g., one of the following sugar moieties: arabinose, 2-fucopyranose, xylose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one of the following modified phosphate backbones: a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkylphosphonate, a formacetal, or an analog of formacetal.

In yet another embodiment, the oligonucleotide is an α-enomeric oligonucleotide. An α-enomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gauier et al, 1987, *Nucl. Acids Res.* 15:6625-6641).

5 The oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent.

Oligonucleotides of the invention may be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biostar, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al, (*Nucl. Acids Res.* (1988) 16:3209), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al, *Proc. Natl. Acad. Sci. USA* (1988) 85:7448-7451).

In a specific embodiment, the SPI antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the SPI antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the SPI antisense RNA can be by any promoter known in the art to act in mammal, preferably human, cells. Such promoters can be inducible or constitutive. Examples of such promoters are outlined above.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene encoding an SPI, preferably a human gene encoding an SPI. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize under stringent conditions (e.g., highly stringent conditions comprising hybridization in 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65 °C and washing in 0.1×SSC/0.1% SDS at 68 °C, or moderately stringent conditions comprising washing in 0.2×SSC/0.1% SDS at 42 °C) with the RNA, forming a stable duplex; in the case of double-stranded SPI antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the

antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA encoding an SPI it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

#### 5.14.6 Therapeutic Use of SPI Antisense Nucleic Acids

The SPI antisense nucleic acids can be used to treat or prevent Schizophrenia when the target SPI is overexpressed in the CSF of subjects suspected of having or suffering from Schizophrenia. In a preferred embodiment, a single-stranded DNA antisense SPI oligonucleotide is used.

Cell types which express or overexpress RNA encoding an SPI can be identified by various methods known in the art. Such cell types include but are not limited to leukocytes (e.g., neutrophils, macrophages, monocytes) and resident cells (e.g., astrocytes, glial cells, neuronal cells, and ependymal cells). Such methods include, but are not limited to, hybridization with an SPI-specific nucleic acid (e.g., by Northern hybridization, dot blot hybridization, *in situ* hybridization), observing the ability of RNA from the cell type to be translated *in vitro* into an SPI, immunocassay, etc. In a preferred aspect, primary tissue from a subject can be assayed for SPI expression prior to treatment, e.g., by immunocytochemistry or *in situ* hybridization.

Pharmaceutical compositions of the invention, comprising an effective amount of an SPI antisense nucleic acid in a pharmaceutically acceptable carrier, can be administered to a subject having Schizophrenia.

The amount of SPI antisense nucleic acid which will be effective in the treatment of Schizophrenia can be determined by standard clinical techniques.

In a specific embodiment, pharmaceutical compositions comprising one or more SPI antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, such compositions may be used to achieve sustained release of the SPI antisense nucleic acids.

#### 5.14.7 Inhibitory Ribozyme and Triple Helix Approaches

In another embodiment, symptoms of Schizophrenia may be ameliorated by decreasing the level of an SPI or SPI activity by using gene sequences encoding the SPI in conjunction with well-known gene "knock-out" ribozyme or triple helix methods to decrease gene expression of an SPI. In this approach ribozyme or triple helix molecules are used to modulate the activity, expression or synthesis of the gene encoding the SPI, and thus to ameliorate the symptoms of Schizophrenia. Such molecules may be designed to reduce or

inhibit expression of a mutant or non-mutant target gene. Techniques for the production and use of such molecules are well known to those of skill in the art.

Ribozyme molecules designed to catalytically cleave gene mRNA transcripts encoding an SPI can be used to prevent translation of target gene mRNA and, therefore, expression of the gene product. (See, e.g., PCT International Publication WO90/11364, published October 4, 1990; Server et al, *Science* (1990) 247:1222-1225).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see Rossi, *Current Biology* (1994) 4:469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, e.g., U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs encoding an SPI, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Myers, 1995, *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff and Gerlach, 1988, *Nature*, 334, 585-591, each of which is incorporated herein by reference in its entirety.

Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA encoding the SPI, i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and that has been extensively described by Thomas Cech and collaborators (Zaug, et al, *Science*, (1984) 224:574-578; Zaug and Cech, *Science*, (1986) 231, 470-475; Zaug, et al, *Nature*, (1986) 324, 429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Ben and Cech, *Cell*, (1986) 47:207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in the gene encoding the SPI.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.) and should be delivered to cells that express the SPI *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous mRNA encoding the SPI and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficacy.

Endogenous SPI expression can also be reduced by inactivating or "knocking out" the gene encoding the SPI, or the promoter of such a gene, using targeted homologous recombination (e.g., see Smithies, et al, *Nature* (1985) 317:230-234; Thomas and Capocchi, *Cell* (1987) 51:503-512; Thompson et al, *Cell* (1989) 5:313-321; and Zijlstra et al, *Nature* (1989) 342:435-438, each of which is incorporated by reference herein in its entirety). For example, a mutant gene encoding a non-functional SPI (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene (either the coding regions or regulatory regions of the gene encoding the SPI) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express the target gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (e.g., see Thomas and Capocchi, 1987 and Thompson, 1989, *supra*). However, this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, the endogenous expression of a gene encoding an SPI can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the gene (i.e., the gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene encoding the SPI in target cells in the body. (See generally, Helene, 1991, *Anticancer Drug Des.*, 6(6):569-584; Helene, et al, *Ann. N.Y. Acad. Sci.*, (1992) 660:27-36; and Maher, *BioEssays* (1992) 14(12):807-815).

Nucleic acid molecules to be used in triple helix formation for the inhibition of transcription should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC+ triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel

orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In instances wherein the antisense, ribozyme, or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique may so efficiently reduce or inhibit the transcription (triple helix) or translation (antisense, ribozyme) of mRNA produced by normal gene alleles of an SPI that the situation may arise wherein the concentration of SPI present may be lower than is necessary for a normal phenotype. In such cases, to ensure that substantially normal levels of activity of a gene encoding an SPI are maintained, gene therapy may be used to introduce into cells nucleic acid molecules that encode and express the SPI that exhibit normal gene activity and that do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. Alternatively, in instances whereby the gene encodes an extracellular protein, normal SPI can be co-administered in order to maintain the requisite level of SPI activity.

Antisense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

#### 5.15 Assays For Therapeutic Or Prophylactic Compounds

The present invention also provides assays for use in drug discovery in order to identify or verify the efficacy of compounds for treatment or prevention of Schizophrenia. Test compounds can be assayed for their ability to restore SF or SPI levels in a subject having

Schizophrenia towards levels found in subjects free from Schizophrenia or to produce similar changes in experimental animal models of Schizophrenia. Compounds able to restore SF or SPI levels in a subject having Schizophrenia towards levels found in subjects free from Schizophrenia or to produce similar changes in experimental animal models of Schizophrenia can be used as lead compounds for further drug discovery, or used therapeutically. SF and SPI expression can't be assayed by the Preferred Technology, immunassays, gel electrophoresis followed by visualization, detection of SPI activity, or any other method taught herein or known to those skilled in the art. Such assays can be used to screen candidate drugs, in clinical monitoring or in drug development, where abundance of an SF or SPI can serve as a surrogate marker for clinical disease.

In various specific embodiments, *in vitro* assays can be carried out with cells representative of cell types involved in a subject's disorder, to determine if a compound has a desired effect upon such cell types.

Compounds for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used. Examples of animal models of Schizophrenia include, but are not limited to, Phencyclidine treated rodents (Sams- Dodd *Rev Neurosci* (1999) 10:59-90), an animal model of deficient sensorimotor gating (Swerdlow and Goyer *Schizoph Bull* (1998) 24(2):285-301), nematal insult the hippocampal region (Buresjö and Bachevalier *Can J Psychiatry* (1996) Sep 41(7):446-56), models based on neonatal excitotoxic hippocampal damage (Lillrank et al., *Clin Neurosci* (1995) 3(2):98-104), attention deficit models (Feldon et al., *J Psychiatr Res* 4:345-66) and NMDA deficient rodent models (Mohn et al., *Cell* 1999, 98, 427-436), animals that show decreased expression of mRNAs for synaptophysin, GAP-43, cholecystokinin, and non-NMDA glutamate receptor subunits (GLU R 1 and 2), particularly in CA 3-4 associated with Schizophrenia (Weinberger *Biol Psychiatry* (1999) Feb 15 45:4 395-402) can be utilized to test compounds that modulate SF or SPI levels, since the neuropathology exhibited in these models is similar to that of Schizophrenia. It is also apparent to the skilled artisan that, based upon the present disclosure, transgenic animals can be produced with "knock-out" mutations of the gene or genes encoding one or more SPIs. A "knock-out" mutation of a gene is a mutation that causes the mutated gene to not be expressed, or expressed in an aberrant form or at a low level, such that the activity associated with the gene product is nearly or entirely absent. Preferably, the transgenic animal is a mammal, more preferably, the transgenic animal is a mouse.

In one embodiment, test compounds that modulate the expression of an SPI are identified in non-human animals (e.g., mice, rats, monkeys, rabbits, and guinea pigs), preferably non-human animal models for Schizophrenia, expressing the SPI. In accordance

with this embodiment, a test compound or a control compound is administered to the animals, and the effect of the test compound on expression of one or more SPIs is determined. A test compound that alters the expression of an SPI (or a plurality of SPIs) can be identified by comparing the level of the selected SPI or SPIs (or mRNA(s) encoding the same) in an animal or group of animals treated with a test compound with the level of the SPI(s) or mRNA(s) in an animal or group of animals treated with a control compound. Techniques known to those of skill in the art can be used to determine the mRNA and protein levels, for example, *in situ* hybridization. The animals may or may not be sacrificed to assay the effects of a test compound.

In another embodiment, test compounds that modulate the activity of an SPI or a biologically active portion thereof are identified in non-human animals (e.g., mice, rats, monkeys, rabbits, and guinea pigs), preferably non-human animal models for Schizophrenia, expressing the SPI. In accordance with this embodiment, a test compound or a control compound is administered to the animals, and the effect of a test compound on the activity of an SPI is determined. A test compound that alters the activity of an SPI (or a plurality of SPIs) can be identified by assaying animals treated with a control compound and animals treated with the test compound. The activity of the SPI can be assessed by detecting induction of a cellular second messenger of the SPI (e.g., intracellular Ca<sup>2+</sup>, diacylglycerol, IP<sub>3</sub>, etc.), detecting catalytic or enzymatic activity of the SPI or binding partner thereof, detecting the induction of a reporter gene (e.g., a regulatory element that is responsive to an SPI of the invention operably linked to a nucleic acid encoding a detectable marker, such as luciferase or green fluorescent protein), or detecting a cellular response (e.g., cellular differentiation or cell proliferation). Techniques known to those of skill in the art can be utilized to detect changes in the activity of an SPI (see, e.g., U.S. Patent No. 5,401,639, which is incorporated herein by reference).

In yet another embodiment, test compounds that modulate the level or expression of an SPI (or plurality of SPIs) are identified in human subjects having Schizophrenia, preferably those having Schizophrenia and most preferably those having severe Schizophrenia. In accordance with this embodiment, a test compound or a control compound is administered to the human subject, and the effect of a test compound on SPI expression is determined by analyzing the expression of the SPI or the mRNA encoding the same in a biological sample (e.g., CSF, serum, plasma, or urine). A test compound that alters the expression of an SPI can be identified by comparing the level of the SPI or mRNA encoding the same in a subject or group of subjects treated with a control compound to that in a subject or group of subjects treated with a test compound. Alternatively, alterations in the expression of an SPI can be identified by comparing the level of the SPI or mRNA encoding the same in a subject or group of subjects before and after the administration of a test compound. Techniques known to those of skill in the art can be used to detect changes in the induction of a second messenger of an SPI or changes in a cellular response.

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to those of skill in the art can be used to obtain the biological sample and analyze the mRNA or protein expression. For example, the Preferred Technology described herein can be used to assess changes in the level of an SPI.

In another embodiment, test compounds that modulate the activity of an SPI (or plurality of SPIs) are identified in human subjects having Schizophrenia, preferably those having Schizophrenia and most preferably those with severe Schizophrenia. In this embodiment, a test compound or a control compound is administered to the human subject, and the effect of a test compound on the activity of an SPI is determined. A test compound that alters the activity of an SPI can be identified by comparing biological samples from subjects treated with a control compound to samples from subjects treated with the test compound. Alternatively, alterations in the activity of an SPI can be identified by comparing the activity of an SPI in a subject or group of subjects before and after the administration of a test compound. The activity of the SPI can be assessed by detecting in a biological sample (e.g., CSF, serum, plasma, or urine) induction of a cellular signal transduction pathway of the SPI (e.g., intracellular Ca<sup>2+</sup>, diacylglycerol, IP<sub>3</sub>, etc.), catalytic or enzymatic activity of the SPI or a binding partner thereof, or a cellular response, for example, cellular differentiation, or cell proliferation. Techniques known to those of skill in the art can be used to detect changes in the induction of a second messenger of an SPI or changes in a cellular response. For example, RT-PCR can be used to detect changes in the induction of a cellular second messenger.

In a preferred embodiment, a test compound that changes the level or expression of an SPI towards levels detected in control subjects (e.g., humans free from Schizophrenia) is selected for further testing or therapeutic use. In another preferred embodiment, a test compound that changes the activity of an SPI towards the activity found in control subjects (e.g., humans free from Schizophrenia) is selected for further testing or therapeutic use.

In another embodiment, test compounds that reduce the severity of one or more symptoms associated with Schizophrenia are identified in human subjects having Schizophrenia, preferably subjects having Schizophrenia and most preferably subjects with severe Schizophrenia. In accordance with this embodiment, a test compound or a control compound is administered to the subjects, and the effect of a test compound on one or more symptoms of Schizophrenia is determined. A test compound that reduces one or more symptoms can be identified by comparing the subjects treated with a control compound to the subjects treated with the test compound. Techniques known to physicians familiar with Schizophrenia can be used to determine whether a test compound reduces one or more symptoms associated with Schizophrenia. For example, a test compound that enhances memory or reduces confusion in a subject having Schizophrenia will be beneficial for treating subjects having Schizophrenia.

In a preferred embodiment, a test compound that reduces the severity of one or more symptoms associated with Schizophrenia in a human having Schizophrenia is selected for further testing or therapeutic use.

#### 5.16 Therapeutic and Prophylactic Compositions and Their Use

The invention provides methods of treatment (and prophylaxis) comprising administering to a subject an effective amount of a compound of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. In a specific embodiment, a non-human mammal is the subject.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid are described above; additional appropriate formulations and routes of administration are described below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticulates, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, *J. Biol. Chem.* (1987) 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction can be enteral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, e.g., by injection, by means of a catheter, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as stialastic

membranes, or fibers. In one embodiment, administration can be by direct injection into CSF or at the site (or former site) of neurodegeneration or to CNS tissue.

In another embodiment, the compound can be delivered in a vesicle, in particular a liposome (see Langer, *Science* (1990) 249:1527-1533; Trent et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, *Surgery* 88:507; Szwedek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.); CRC Press, Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* (1983) 23:61; see also Levy et al., *Science* (1985) 238:190; During et al., *Ann. Neurol.* (1989) 25:351; Howard et al., *J. Neurosurg.* (1989) 71:105.). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodison, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* (1990) 249:1527-1533).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection or by use of microparticle bombardment (e.g., a gene gun; Biostatic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers

can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agent. These compositions can take the form of solutions, suspensions, emulsion tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile, isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachet indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment of Schizophrenia can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects (a) approval by the agency of manufacture, use or sale for human administration, (b) directions for use, or both.

## 6. EXAMPLE: IDENTIFICATION OF PROTEINS DIFFERENTIALLY EXPRESSED IN THE CSF IN SCHIZOPHRENIA

Using the following procedure, proteins in CSF samples from 5 subjects having Schizophrenia and 5 control subjects were separated by isoelectric focusing followed by SDS-PAGE and analyzed. Parts 6.1.1 to 6.1.14 (inclusive) of the procedure set forth are hereby designated as the "Reference Protocol"

### 6.1 MATERIALS AND METHODS

#### 6.1.1 Sample Preparation

A protein assay (Pierce BCA Cat # 23225) was performed on each CSF sample as received. Prior to protein separation, each sample was processed for selective depletion of certain proteins, in order to enhance and simplify protein separation and facilitate analysis by removing proteins that may interfere with or limit analysis of proteins of interest. See International Patent Application No. PCT/GB99/01742, filed June 1, 1999, which is incorporated by reference in its entirety; with particular reference to pages 3 and 6.

Removal of albumin, haptoglobin, transferrin and immunoglobulin G (IgG) from CSF ("CSF depletion") was achieved by an affinity chromatography purification step in which the

sample was passed through a series of "Hi-Trap" columns containing immobilized antibodies for selective removal of albumin, haptoglobin and transferrin, and protein G for selective removal of immunoglobulin G. Two affinity columns in a tandem assembly were prepared by coupling antibodies to protein G-sepharose contained in Hi-Trap columns (Protein G-Sepharose Hi-Trap columns (1 ml) Pharmacia Cat. No. 17-0404-01). This was done by circulating the following solutions sequentially through the columns: (1) Dulbecco's Phosphate Buffered Saline (Gibco BRL Cat. No. 14109-094); (2) concentrated antibody solution; (3) 200 mM sodium carbonate buffer, pH 8.35; (4) cross-linking solution (200 mM sodium carbonate buffer, pH 8.35, 20 mM dimethylpimelidate); and (5) 500 mM ethanolamine, 500 mM NaCl. A third (un-derivatized) protein G Hi-Trap column was then attached to the lower end of the tandem column assembly.

The chromatographic procedure was automated using an Akta Fast Protein Liquid Chromatography (FPLC) System such that a series of up to seven runs could be performed sequentially. The samples were passed through the series of 3 Hi-Trap columns in which the affinity chromatography media selectively bind the above proteins thereby removing them from the sample. Fractions (typically 3 ml per tube) were collected of unbound material ("Flowthrough fractions") that eluted through the column during column loading and washing stages and of bound protein ("Bound/Eluted fractions") that were eluted by step elution with Immunopure Gentle Ag/Ab Elution Buffer (Pierce Cat. No. 21013). The eluate containing unbound material was collected in fractions which were pooled, desalinated/concentrated by centrifugal ultrafiltration and stored to await further analysis by 2D PAGE.

A volume of depleted CSF containing approximately 100-150 µg of total protein was aliquoted and an equal volume of 10% (w/v) SDS (Fluka 71729), 2.3% (w/v) dithiothreitol (BDH 443852A) was added. The sample was heated at 95 °C for 5 mins, and then allowed to cool to 20 °C. 125 µl of the following buffer was then added to the sample:

8M urea (BDH 452043w)  
4% CHAPS (Sigma C3023)  
65mM dithiothreitol (DTT)  
2% (v/v) Resolutes 3.5-10 (BDH 44338 2x) This mixture was vortexed, and centrifuged at 13000 rpm for 5 mins at 15 °C, and the supernatant was analyzed by isoelectric focusing.

#### 6.1.2 Isoelectric Focusing

Isoelectric focusing (IEF), was performed using the Immobiline® DryStrip Kit (Pharmacia BioTech), following the procedure described in the manufacturer's instructions, see Instructions for Immobiline® DryStrip Kit, Pharmacia, # 18-1038-63, Edition AB

(incorporated herein by reference in its entirety). Immobilized pH Gradient (IPG) strips (18cm, pH 3-10 non-linear strips; Pharmacia Cat. # 17-1235-01) were rehydrated overnight at 20 °C in a solution of 6M urea, 2% (w/v) CHAPS, 10mM DTT, 2% (v/v) Resolytes 3.5-10, as described in the Immobilized DryStrip Users Manual. For IEF, 50 µl of supernatant (prepared as above) was loaded onto a strip, with the cap-loading units being placed at the basic end of the strip. The loaded gels were then covered with mineral oil (Pharmacia 17-3335-01) and a voltage was immediately applied to the strips according to the following profile, using a Pharmacia EPS3500XL power supply (Cat 19-3500-01):

Initial voltage = 300V for 2 hrs

Linear Ramp from 300V to 3500V over 3hrs

Hold at 3500V for 19hrs For all stages of the process, the current limit was set to 10mA for 12 gels, and the wattage limit to 5W. The temperature was held at 20 °C throughout the run.

#### 6.1.3 Gel Equilibration and SDS-PAGE

After the final 19hr step, the strips were immediately removed and immersed for 10 mins at 20 °C in a first solution of the following composition: 6M urea; 2% (w/v) DTT; 2% (w/v) SDS; 30% (v/v) glycerol (Fluka 49767); 0.05M Tris/HCl, pH 6.8 (Sigma Cat T-1503). The strips were removed from the first solution and immersed for 10 mins at 20 °C in a second solution of the following composition: 6M urea; 2% (w/v) iodoacetamide (Sigma I-6125); 2% (w/v) SDS; 30% (v/v) glycerol; 0.05M Tris/HCl, pH 6.8. After removal from the second solution, the strips were loaded onto supported gels for SDS-PAGE according to Hochstrasser et al, 1988, Analytical Biochemistry 173: 412-423 (incorporated herein by reference in its entirety), with modifications as specified below:

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#### 6.1.4 Preparation of supported gels

The gels were cast between two glass plates of the following dimensions: 23cm wide x 24cm long (back plate); 23cm wide x 24cm long with a 2cm deep notch in the central 19cm (front plate). To promote covalent attachment of SDS-PAGE gels, the back plate was treated with a 0.4% solution of  $\gamma$ -methacryloyloxypropyltrimethoxysilane in ethanol (BindSilane<sup>TM</sup>; Pharmacia Cat. # 17-1330-01). The front plate was treated with (RepelSilane<sup>TM</sup> Pharmacia Cat. # 17-1332-01) to reduce adhesion of the gel. Excess reagent was removed by washing with water, and the plates were allowed to dry. At this stage, both as identification for the gel, and as a marker to identify the coated face of the plate, an adhesive bar-code was attached to the back plate in a position such that it would not come into contact with the gel matrix.

Upon completion of the electrophoresis run, the gels were immediately removed from the tank for fixation. The top plate of the gel cassette was carefully removed, leaving the gel bonded to the bottom plate. The bottom plate with its attached gel was then placed into a staining apparatus, which can accommodate 12 gels. The gels were completely immersed in a fixative solution of 40% (v/v) ethanol (BDH 28719), 10% (v/v) acetic acid (BDH 100016X), 50% (v/v) water (Millipore), which was continuously circulated over the gels. After an overnight incubation, the fixative was drained from the tank, and the gels were primed by immersion in 7.5% (v/v) acetic acid, 0.05% (w/v) SDS, 92.5% (v/v) water for 30 mins. The priming solution was then drained, and the gels were stained by complete immersion for 4 hours in a staining solution of Pyridinium, 4-[2-(4-dimethylamino)-2-trifluoromethylphenyl]ethenyl]-1-(subbutyloxy)-, inner salt, prepared by diluting a stock solution of this dye (2mg/ml in DMSO) in 1.5% (v/v) aqueous acetic acid to give a final concentration of 1.2 mg/l; the staining solution was vacuum filtered through a 0.4µm filter (Duropore) before use.

#### 6.1.7 Imaging of the gel

A computer-readable output was produced by imaging the fluorescently stained gels with the Apollo 2 scanner (Oxford Glycosciences, Oxford, UK) described in section 5.1, supra. This scanner has a gel carrier with four integral fluorescent markers (Designated M1, M2, M3, M4) that are used to correct the image geometry and are a quality control feature to confirm that the scanning has been performed correctly.

For scanning, the gels were removed from the stain, rinsed with water and allowed to air dry briefly, and imaged on the Apollo 2. After imaging, the gels were sealed in polyethylene bags containing a small volume of staining solution, and then stored at 4 °C.

#### 6.1.8 Digital Analysis of the Data

The data were processed as described in U.S. Application Serial No. 08/980,574, (published as WO 98/23950) at Sections 5.4 and 5.5 (incorporated herein by reference), as set forth more particularly below.

The output from the scanner was first processed using the MELANIE<sup>®</sup> II 2D PAGE analysis program (Release 2.2, 1997, BioRad Laboratories, Hercules, California, Cat. # 170-7566) to autodetect the registration points, M1, M2, M3 and M4; to autocrop the images (i.e., to eliminate signals originating from areas of the scanned image lying outside the boundaries of the gel, e.g. the reference frame); to filter out artifacts due to dust; to detect and quantify features; and to create image files in GIF format. Features were detected using the following parameters:

Smooths = 2

Laplacian threshold 50

The dried plates were assembled into a casting box with a capacity of 13 gel sandwiches. The top and bottom plates of each sandwich were spaced by means of 1mm thick spacers, 2.5 cm wide. The sandwiches were interleaved with acetate sheets to facilitate separation of the sandwiches after gel polymerization. Casting was then carried out according to Hochstrasser et al, op. cit.

A 9-16% linear polyacrylamide gradient was cast, extending up to a point 2cm below the level of the notch in the front plate, using the Angelique gradient casting system (Large Scale Biology). Stock solutions were as follows. Acrylamide (40% in water) was from Serva (Cat. # 10677). The cross-linking agent was PDA (BioRad 161-0202), at a concentration of 2.6% (w/w) of the total starting monomer content. The gel buffer was 0.375M Tris/HCl, pH 8.8. The polymerization catalyst was 0.05% (v/v) TEMED (BioRad 161-0801), and the initiator was 0.1% (w/v) APS (BioRad 161-0700). No SDS was included in the gel and no stacking gel was used. The cast gels were allowed to polymerize at 20 °C overnight, and then stored at 4 °C in sealed polyethylene bags with 6ml of gel buffer, and were used within 4 weeks.

#### 6.1.5 SDS-PAGE

A solution of 0.5% (w/v) agarose (Fluka Cat 05075) was prepared in running buffer (0.025M Tris, 0.193M glycine (Fluka 50050), 1% (w/v) SDS, supplemented by a trace of bromophenol blue). The agarose suspension was heated to 70 °C with stirring, until the agarose had dissolved. The top of the supported 2nd D gel was filled with the agarose solution, and the equilibrated strip was placed into the agarose, and tapped gently with a palette knife until the gel was intimately in contact with the 2nd D gel. The gels were placed in the 2nd D running tank, as described by Ames et al, 1995, Electrophoresis 16: 1255-1267 (incorporated herein by reference in its entirety). The tank was filled with running buffer (as above) until the level of the buffer was just higher than the top of the region of the 2nd D gels which contained polyacrylamide, so as to achieve efficient cooling of the active gel area. Running buffer was added to the top buffer compartments formed by the gels, and then voltage was applied immediately to the gels using a Consort B-833 power supply. For 1 hour, the gels were run at 20mA/gel. The wattage limit was set to 150W for a tank containing 6 gels, and the voltage limit was set to 600V. After 1 hour, the gels were then run at 40mA/gel, with the same voltage and wattage limits as before, until the bromophenol blue line was 0.5cm from the bottom of the gel. The temperature of the buffer was held at 16 °C throughout the run. Gels were not run in duplicate.

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#### 6.1.6 Staining

Partials threshold 1  
Saturation = 100  
Peakdean = 0  
Minimum Perimeter = 10

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#### 6.1.9 Assignment of pI and MW Values

Landmark identification was used to determine the pI and MW of features detected in the images. Twelve landmark features, designated CSF1 to CSF12, were identified in a standard CSF image obtained from a pooled sample. These landmark features are identified in Figure 1 and were assigned the pI and/or MW values identified in Table XV.

Name	pI	MW (Da)	Name	pI	MW (Da)
CSF1	5.96	185230	CSF7	4.78	41340
CSF2	5.39	141700	CSF8	9.2	40000
CSF3	5.29	100730	CSF9	5.5	31800
CSF4	5.08	71270	CSF10	6.94	27440
CSF5	7.68	68370	CSF11	5.8	23890
CSF6	5.57	48080	CSF12	6.43	10950

As many of these landmarks as possible were identified in each gel image of the dataset. Each feature in the study gels was then assigned a pI value by linear interpolation or extrapolation (using the MELANIE<sup>®</sup> II software) to the two nearest landmarks, and was assigned a MW value by linear interpolation or extrapolation (using the MELANIE<sup>®</sup> II software) to the two nearest landmarks.

#### 6.1.10 Matching With Primary Master Image

Images were edited to remove gross artifacts such as dust, to reject images which had gross abnormalities such as smearing of protein features, or were of too low a loading or overall image intensity to allow identification of more than the most intense features, or were of too poor a resolution to allow accurate detection of features. Images were then compared by pairing with one common image from the whole sample set. This common image, the "primary master image", was selected on the basis of protein load (maximum load consistent with maximum feature detection), a well resolved myoglobin region, (myoglobin was used as an internal standard), and general image quality. Additionally, the primary master image was chosen to be an image which appeared to be generally representative of all those to be included in the analysis. (This process by which a primary master gel was judged to be

representative of the study gels was rechecked by the method described below and in the event that the primary master gel was seen to be unrepresentative, it was rejected and the process repeated until a representative primary master gel was found.)

Each of the remaining study gel images was individually matched to the primary master image such that common protein features were paired between the primary master image and each individual study gel image as described below.

#### 6.1.11 Cross-matching Between Samples

To facilitate statistical analysis of large numbers of samples for purposes of identifying features that are differentially expressed, the geometry of each study gel was adjusted for maximum alignment between its pattern of protein features, and that of the primary master, as follows. Each of the study gel images was individually transformed into the geometry of the primary master image using a multi-resolution warping procedure. This procedure corrects the image geometry for the distortions brought about by small changes in the physical parameters of the electrophoresis separation process from one sample to another. The observed changes are such that the distortions found are not simple geometric distortions, but rather a smooth flow, with variations at both local and global scale.

The fundamental principle in multi-resolution modeling is that smooth signals may be modeled as an evolution through 'scale space', in which details at successively finer scales are added to a low resolution approximation to obtain the high resolution signal. This type of model is applied to the flow field of vectors (defined at each pixel position on the reference image) and allows flows of arbitrary smoothness to be modeled with relatively few degrees of freedom. Each image is first reduced to a stack, or pyramid, of images derived from the initial image, but smoothed and reduced in resolution by a factor of 2 in each direction at every level (Gaussian pyramid) and a corresponding difference image is also computed at each level, representing the difference between the smoothed image and its progenitor (Laplacian pyramid). Thus the Laplacian images represent the details in the image at different scales.

To estimate the distortion between any 2 given images, a calculation was performed at level 7 in the pyramid (*i.e.* after 7 successive reductions in resolution). The Laplacian images were segmented into a grid of 16x16 pixels, with 50% overlap between adjacent grid positions in both directions, and the cross correlation between corresponding grid squares on the reference and the test images was computed. The distortion displacement was then given by the location of the maximum in the correlation matrix. After all displacements had been calculated at a particular level, they were interpolated to the next level in the pyramid, applied to the test image, and then further corrections to the displacements were calculated at the next scale.

After matching all component gels in the study to the final composite master image, the intensity of each feature was measured and stored. The end result of this analysis was the generation of a digital profile which contained, for each identified feature: 1) a unique identification code relative to corresponding feature within the composite master image (MCI), 2) the x, y coordinates of the features within the gel, 3) the isoelectric point (pI) of the SFs, 4) the apparent molecular weight (MW) of the SFs, 5) the signal value, 6) the standard deviation for each of the preceding measurements, and 7) a method of linking the MCIs of each feature to the master gel to which this feature was matched. By virtue of a Laboratory Information Management System (LIMS), this MCI profile was traceable to the actual stored gel from which it was generated, so that proteins identified by computer analysis of gel profile databases could be retrieved. The LIMS also permitted the profile to be traced back to an original sample or patient.

#### 6.1.13 Statistical Analysis of the Profiles

The complementary statistical strategies specified below were used in the order in which they are listed to identify SFs from the MCIs within the mastergroup.

(a) The Wilcoxon Rank-Sum test. This test was performed between the control and the Schizophrenia samples for each MCI basis. The MCIs which recorded a p-value less than or equal to 0.05 were selected as statistically significant SFs with 95% selectivity.

(b) A second non-overlapping selection strategy is based on the fold change. A fold change representing the ratio of the average normalized protein abundances of the SFs within an MCI was calculated for each MCI between each set of controls and Schizophrenia samples. An 80% confidence limit for the mean of the fold changes was calculated. The MCIs with fold changes which fall outside the confidence limit were selected as SFs which met the criteria of the significant fold change threshold with 80% selectivity. Because the MCI fold changes are based on a 80% confidence limit, it follows that the significant fold change threshold is itself 80%.

(c) A third non-overlapping selection strategy is based on qualitative presence or absence alone. Using this procedure, a percentage feature presence was calculated across the control samples and Schizophrenia samples for each MCI which was a potential SF based on such qualitative criteria alone, *i.e.* presence or absence. The MCIs which recorded a percentage feature presence of 80% or more on Schizophrenia samples and a percentage feature presence of 20% or less on control samples, were selected as the qualitative differential SFs with 80% selectivity. A second group of qualitative differential SFs with 80% selectivity were formed by those MCIs which recorded a percentage feature presence of 80% or more on control samples and a percentage feature presence of 20% or less on Schizophrenia samples.

The warping process brought about good alignment between the common features in the primary master image, and the images for the other samples. The MELANIE® II 2D PAGE analysis program was used to calculate and record approximately 500-700 matched feature pairs between the primary master and each of the other images. The accuracy of this program was significantly enhanced by the alignment of the images in the manner described above. To improve accuracy still further, all pairings were finally examined by eye in the McView interactive editing program and residual recognizably incorrect pairings were removed. Where the number of such recognizably incorrect pairings exceeded the overall reproducibility of the Preferred Technology (as measured by repeat analysis of the same biological sample) the gel selected to be the primary master gel was judged to be insufficiently representative of the study gels to serve as a primary master gel. In that case, the gel chosen as the primary master gel was rejected, and different gel was selected as the primary master gel, and the process was repeated.

All the images were then added together to create a composite master image, and the positions and shapes of all the gel features of all the component images were super-imposed onto this composite master as described below.

Once all the initial pairs had been computed, corrected and saved, a second pass was performed whereby the original (unwarped) images were transformed a second time to the geometry of the primary master, this time using a flow field computed by smooth interpolation of the multiple tie-points defined by the centroids of the paired gel features. A composite master image was thus generated by initialising the primary master image with its feature descriptors. As each image was transformed into the primary master geometry, it was digitally summed pixel by pixel into the composite master image, and the features that had not been paired by the procedure outlined above were likewise added to the composite master image description, with their centroids adjusted to the master geometry using the flow field correction.

The final stage of processing was applied to the composite master image and its feature descriptors, which now represent all the features from all the images in the study transformed to a common geometry. The features were grouped together into linked sets or "clusters", according to the degree of overlap between them. Each cluster was then given a unique identifying index, the molecular cluster index (MCI).

An MCI identifies a set of matched features on different images. Thus an MCI represents a protein or proteins eluting at equivalent positions in the 2D separation in different samples.

#### 6.1.12 Construction of Profiles

Application of these three analysis strategies allowed SFs to be selected on the basis of: (a) statistical significance as measured by the Wilcoxon Rank-Sum test, (b) a significant fold change threshold with a chosen selectivity, or (c) qualitative differences with a chosen selectivity.

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#### 6.1.14 Recovery and analysis of selected proteins

Proteins in SFs were robotically excised and processed to generate tryptic digest peptides. Tryptic peptides were analyzed by mass spectrometry using a PerSeptive Biosystems Voyager-DETM STR Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometer, and selected tryptic peptides were analyzed by tandem mass spectrometry (MS/MS) using a Micromass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (Micromass, Altrincham, U.K.) equipped with a nanoflow™ electrospray Z-spray source. For partial amino acid sequencing and identification of SFs uninterpreted tandem mass spectra of tryptic peptides were searched using the SEQUEST search program (Eng et al, 1994, J. Am. Soc. Mass Spectrom. 5:976-989), version v.C.1. Criteria for database identification included: the cleavage specificity of trypsin; the detection of a suite of a, b and y ions in peptide returned from the database, and a mass increment for all Cys residues to account for carbamidomethylation. The database searched was database constructed of protein entries in the non-redundant database held by the National Centre for Biotechnology Information (NCBI) which is accessible at <http://www.ncbi.nlm.nih.gov/>. Following identification of proteins through spectral-spectral correlation using the SEQUEST program, masses detected in MALDI-TOF mass spectra were assigned to tryptic digest peptides within the proteins identified. In cases where no amino acid sequences could be identified through searching with uninterpreted MS/MS spectra of tryptic digest peptides using the SEQUEST program, tandem mass spectra of the peptides were interpreted manually, using methods known in the art. (In the case of interpretation of low-energy fragmentation mass spectra of peptide ions see Gaskell et al, 1992, Rapid Commun. Mass Spectrom. 6:658-662)

#### EXAMPLE: DIAGNOSIS AND TREATMENT OF SCHIZOPHRENIA

The following example illustrates the use of a SPI of the invention for screening or diagnosis of Schizophrenia, determining the prognosis of a Schizophrenia patient, or monitoring the effectiveness of Schizophrenia therapy. The following example also illustrates the use of modulators (*e.g.*, agonist or antagonists) of a SPI of the invention to treat or prevent Schizophrenia.

Neuronal pentraxins (NP-1 and NP-2) are expressed predominantly in the nervous system and belong to the pentraxin protein family that also includes serum amyloid P

component (AP) and C-reactive protein (CRP) (Schlingens et al., 1995) *Neuron* 3, 519-526; Omieis LA, et al., (1996) *Genomics* 36,543-545) and neuronal activity-regulated pentraxin (NARP) (Tsui et al., (1996) *J Neurosci* 16, 2463-2478). NP-1 and NP-2 have a putative role in the uptake of synaptic macromolecules during synaptogenesis and plasticity (Dodds DC et al., 1997) *J Biol Chem* 272, 21488-94). Addition of NP-1 to glial cultures renders them susceptible to taipoxin toxicity (Dodds DC et al., *supra*). The expression of NP-1 (SF-223, SPI-118) has been shown herein to be significantly increased in the cerebrospinal fluid (CSF) of subjects having Schizophrenia as compared with the CSF of subjects free from Schizophrenia (see Table II). Thus, quantitative detection of NP-1 in CSF can be used to diagnose Schizophrenia, determine the progression of Schizophrenia or monitor the effectiveness of a therapy for Schizophrenia.

We also identified a putative human protein (SPI-206), the neuronal pentraxin receptor (hNPR), whose gene is located on chromosome 22q12.3-13.2 (accession ID AL008583), an ortholog of the rat neuronal pentraxin receptor (rNPR) (Kirkpatrick LL et al., (2000) *J Biol Chem* 275, 17786-92; Dodds DC et al., *supra*). SPI-206 is 87% identical at the peptide sequence level to rNPR. The rat protein, a putative integral membrane pentraxin, has 49 and 48% identity to neuronal pentraxin 1 and neuronal pentraxin 2, respectively (Dodds DC et al., *supra*). rNPR is expressed on the cell membrane and can form heteropentamers with NP-1 and NP-2 that can be released from cell membranes (Kirkpatrick et al., *supra*). hNPR (SPI-206) has 48% homology with serum amyloid P, a protein that has been described for its role in amyloid deposition, which is delayed in mice with targeted deletion of the serum amyloid P component gene (Botto et al. (1997) *Nat Med* 3(8), 855-9). The expression of hNPR (SF-342, SPI-206) has been shown herein to be significantly increased in the cerebrospinal fluid (CSF) of subjects having Schizophrenia as compared with the CSF of subjects free from Schizophrenia (see Table II). Thus, quantitative detection of hNPR in CSF can be used to diagnose Schizophrenia, determine the progression of Schizophrenia or monitor the effectiveness of a therapy for Schizophrenia.

In one embodiment of the invention, compounds that modulate (e.g., upregulate or downregulate) the expression, activity or both the expression and activity of NP-1 or hNPR are administered to a subject in need of treatment or for prophylaxis of Schizophrenia. Antibodies that modulate the expression, activity or both the expression and activity of NP-1 or hNPR are suitable for this purpose. In addition, nucleic acids coding for all or a portion of NP-1 or hNPR, or nucleic acids complementary to all or a portion of NP-1 or hNPR, may be administered. NP-1 or hNPR, or fragments of the NP-1 or hNPR polypeptide may also be administered.

The invention also provides screening assays to identify additional compounds that modulate the expression of NP-1 or activity of NP-1. Compounds that modulate the

expression of NP-1 in vitro can be identified by comparing the expression of NP-1 in cells treated with a test compound to the expression of NP-1 in cells treated with a control compound (e.g., saline). Methods for detecting expression of NP-1 are known in the art and include measuring the level of NP-1 RNA (e.g., by northern blot analysis or RT-PCR) and measuring NP-1 protein (e.g., by immunoassay or western blot analysis). Compounds that modulate the activity of NP-1 can be identified by comparing the ability of a test compound to agonize or antagonize a function of NP-1, such as its effects on synaptogenesis or plasticity or its binding to hNPR, to the ability of a control compound (e.g., saline) to inhibit the same function of NP-1. Compounds capable of modulating NP-1 binding to its receptor or NP-1 activity are identified as compounds suitable for further development as a compound useful for the treatment of Schizophrenia.

Binding between NP-1 and its receptor can be determined by, for example, contacting NP-1 with cells known to express the hNPR and assaying the extent of binding between NP-1 and the hNPR cell surface receptor, or by contacting NP-1 with hNPR in a cell-free assay, i.e., an assay where the NP-1 and hNPR are isolated, and, preferably, recombinantly produced, and assaying the extent of binding between NP-1 and hNPR. Through the use of such assays, candidate compounds may be tested for their ability to agonize or antagonize the binding of NP-1 to hNPR.

Compounds identified in vitro that affect the expression or activity of NP-1 can be tested in vivo in animal models of Schizophrenia, to determine their therapeutic efficacy.

#### EXAMPLE: TISSUE SPECIFIC EXPRESSION OF SPI-206

Real time quantitative RT-PCR (Heid, C.A., Stevens, J., Livak, K.J. & Williams, P.M. Real time quantitative PCR. *Genome Res.* 6, 986-994 (1996); Morrison, T.B., Weis, J.J. & Wittwer, C.T. Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. *Biotechniques* 24, 954- 958 (1998)) was utilized to analyze the distribution of SPI-206 mRNA in normal human tissues (Fig 3). The primers used for PCR were taken from the 3' untranslated region of Genbank entry AL162057, and were as follows: sense, 5' acaccacacatccggatc 3', antisense, 5' trggagggtggatgggggg 3'. Reactions containing 10ng cDNA, SYBR green sequence detection reagents (PE Biosystems) and sense and antisense primers were assayed on an ABI7700 sequence detection system (PE Biosystems). The PCR conditions were 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 10 min, and 40 cycles of 95°C for 15s, 55°C for 1min. The accumulation of PCR product was measured in real time as the increase in SYBR green fluorescence, and the data were analyzed using the Sequence Detector program v1.6.3 (PE Biosystems). Standard curves relating initial template copy number to fluorescence and amplification cycle were generated using the

amplified PCR product as a template, and were used to calculate SPI-206 copy number in each sample.

Figure 3 clearly shows the brain specificity of SPI-206 (up to 2300 copy numbering cDNA), with low systemic levels (maximum 91 copy number/ng cDNA in fetal lung tissue).

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#### EXAMPLE: TISSUE SPECIFIC EXPRESSION OF SPI-238 AND SPI-240

We used real time quantitative RT-PCR (Heid et al., 1996; Morrison et al., 1998) to analyze the distribution of SPI-238 and SPI-240 mRNA in normal human tissues (Figure 5). The distribution of SPI 238/240 mRNA was restricted in the body and elevated in all parts of the brain.

Real time quantitative RT-PCR (Heid, C.A., Stevens, J., Livak, K.J. & Williams, P.M. Real time quantitative PCR. *Genome Res.* 6, 986-994 (1996); Morrison, T.B., Weis, J.J. & Wittwer, C.T. Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. *Biotechniques* 24, 954- 958 (1998)) was utilized to analyze the distribution of SPI 238/240 mRNA in normal human tissues (Figure 5). The primers used for PCR were derived from the SPI-238 and SPI-240 coding region, and were as follows: sense, 5' atggaaatggcgccgttgtt 3', antisense, 5' agatggatggatctccagg 3'. Reactions containing 10ng cDNA, SYBR green sequence detection reagents (PE Biosystems) and sense and antisense primers were assayed on an ABI7700 sequence detection system (PE Biosystems). The PCR conditions were 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 10 min, and 40 cycles of 95°C for 15s, 65°C for 1min. The accumulation of PCR product was measured in real time as the increase in SYBR green fluorescence, and the data were analyzed using the Sequence Detector program v1.6.3 (PE Biosystems). Standard curves relating initial template copy number to fluorescence and amplification cycle were generated using the amplified PCR product as a template, and were used to calculate SPI 238/240 copy number in each sample.

Figure 5 clearly shows the brain specificity of SPI 238/240 (up to 4100 copy number/ng cDNA), with low systemic levels (maximum 83 copy numbering cDNA in liver tissue).

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#### EXAMPLE: PREDICTIVE ANALYSIS OF SPI-238 AND SPI-240

Although the amino acid sequence shown in Figure 4A shares 44% identity with a putative human protein derived from a conceptual translation of the cDNA CAB07646.1 (available at <http://www.ncbi.nlm.nih.gov/cabre2>), no function has been assigned to CAB07646.1. PSORT (Nakai, K. and Kimchiya, M., A knowledge base for predicting protein localization sites in eukaryotic cells, *Genomics* 14, 897-911. (1992)) analysis of the amino

acid sequence shown in Figure 4 identifies only a signal sequence at amino acids 1-20, with proteolytic cleavage predicted between amino acids 20 and 21.

Thus the mRNA expression and protein structure analyses are consistent with this protein being secreted from brain tissues and being assayable in CSF.

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The present invention is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the invention. Functionally equivalent methods and apparatus within the scope of the invention, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications and variations are intended to fall within the scope of the appended claims. The contents of each reference, patent and patent application cited in this application is hereby incorporated by reference in its entirety.

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## WB CLAIM:

1. A method for screening, diagnosis or prognosis of Schizophrenia in a subject, for determining the stage or severity of Schizophrenia in a subject, for identifying a subject at risk of developing Schizophrenia, or for monitoring the effect of therapy administered to a subject having Schizophrenia, said method comprising:

5 (a) analyzing a test sample of body fluid from the subject by two dimensional electrophoresis to generate a two-dimensional array of features, said array comprising at least one chosen feature whose relative abundance correlates with the presence, absence, stage or severity of Schizophrenia or predicts the onset or course of Schizophrenia; and

10 (b) comparing the abundance of each chosen feature in the test sample with the abundance of that chosen feature in body fluid from one or more persons free from Schizophrenia, or with a previously determined reference range for that feature in subjects free from Schizophrenia, or with the abundance at least one Expression Reference Feature (ERF) in the test sample.

15 2. The method of claim 1, wherein the body fluid is cerebrospinal fluid (CSF).

3. The method of claim 1 or claim 2, wherein said method is for screening or 20 diagnosis of Schizophrenia and the relative abundance of at least one chosen feature correlates with the presence or absence of Schizophrenia.

25 4. The method of claim 1 or claim 2, wherein said method is for monitoring the effect of therapy administered to a subject having Schizophrenia and the relative abundance of at least one chosen feature correlates with the severity of Schizophrenia.

30 5. The method of claim 2, wherein step (b) comprises comparing the abundance of each chosen feature in the sample with the abundance of that chosen feature in CSF from one or more persons free from Schizophrenia or with a previously determined reference range for that chosen feature in subjects free from Schizophrenia.

35 6. The method of claim 1 or claim 2, wherein step (b) comprises detecting one or more of the following Schizophrenia-Associated Features (SFs): SF-14, SF-16, SF-17, SF-18, SF-19, SF-20, SF-21, SF-22, SF-23, SF-24, SF-25, SF-26, SF-27, SF-28, SF-29, SF-30, SF-31, SF-32, SF-33, SF-34, SF-35, SF-36, SF-37, SF-38, SF-39, SF-40, SF-41, SF-42, SF-43, SF-44, SF-45, SF-46, SF-47, SF-48, SF-49, SF-51, SF-52, SF-53, SF-55, SF-56, SF-57, SF-58, SF-59, SF-60, SF-61, SF-62, SF-63, SF-64, SF-65, SF-66, SF-67, SF-68, SF-69, SF-70, SF-71, SF-72, SF-73, SF-74, SF-75, SF-76, SF-77, SF-78, SF-79, SF-80, SF-81, SF-82, SF-83, SF-84, SF-85, SF-86, SF-87, SF-88, SF-89, SF-90, SF-

91, SF-92, SF-93, SF-94, SF-95, SF-96, SF-97, SF-98, SF-99, SF-100, SF-101, SF-102, SF-103, SF-104, SF-105, SF-106, SF-107, SF-108, SF-109, SF-110, SF-111, SF-112, SF-113, SF-114, SF-115, SF-116, SF-117, SF-118, SF-119, SF-120, SF-123, SF-124, SF-125, SF-126, SF-130, SF-131, SF-132, SF-135, SF-136, SF-137, SF-139, SF-141, SF-142, SF-143, SF-144,

5 SF-147, SF-148, SF-150, SF-151, SF-153, SF-154, SF-155, SF-157, SF-158, SF-159, SF-160, SF-161, SF-162, SF-163, SF-164, SF-165, SF-166, SF-167, SF-168, SF-169, SF-170, SF-171, SF-172, SF-173, SF-174, SF-175, SF-176, SF-177, SF-178, SF-179, SF-180, SF-181, SF-182, SF-183, SF-184, SF-186, SF-187, SF-188, SF-189, SF-190, SF-191, SF-194, SF-195, SF-196, SF-197, SF-198, SF-199, SF-200, SF-201, SF-202, SF-203, SF-204, SF-208, SF-209, SF-211,

10 SF-212, SF-213, SF-215, SF-216, SF-217, SF-218, SF-219, SF-220, SF-221, SF-222, SF-226, SF-227, SF-228, SF-229, SF-230, SF-231, SF-232, SF-233, SF-235, SF-237, SF-238, SF-239, SF-242, SF-243, SF-244, SF-245, SF-249, SF-250, SF-255, SF-257, SF-258, SF-261, SF-262, SF-264, SF-265, SF-267, SF-268, SF-269, SF-271, SF-272, SF-273, SF-280, SF-281, SF-282, SF-283, SF-286, SF-289, SF-291, SF-292, SF-293, SF-294, SF-296, SF-300, SF-301, SF-302, SF-303,

15 SF-304, SF-306, SF-307, SF-309, SF-312, SF-317, SF-320, SF-321, SF-322, SF-324, SF-326, SF-327, SF-332, SF-333, SF-336, SF-340, SF-348, SF-349, SF-352, SF-358, SF-368, SF-369, SF-370, SF-371, SF-372, SF-373, SF-374, SF-375, SF-376, SF-378, SF-379, SF-380, SF-381, SF-382, SF-383, SF-384, SF-385, SF-386, SF-387, SF-388, SF-389, SF-390, SF-391, SF-392, SF-393, SF-394, SF-395, SF-396, SF-397, SF-398, SF-399, SF-400, SF-401, SF-402,

20 SF-403, SF-404, SF-405, SF-406, SF-407, SF-408, SF-409, SF-410, SF-411, SF-412, SF-413, SF-414, SF-415, SF-416, SF-417, SF-418, SF-419, SF-420, SF-421, SF-422, SF-423, SF-424, SF-425, SF-426, SF-427, SF-428, SF-429, SF-430, SF-431, SF-432, SF-433, SF-434, SF-435, SF-436, SF-437, SF-438, SF-439, SF-440, SF-441, SF-442, SF-443, SF-444, SF-445, SF-446, SF-447, SF-448, SF-449, SF-450, SF-451, SF-452, SF-453, SF-454, SF-455, SF-456, SF-457,

25 SF-458, SF-459, SF-460, SF-461, SF-462, SF-463, SF-464, SF-465, SF-466, SF-467, SF-468, SF-469, SF-470, SF-471, SF-472, SF-473, SF-474, SF-475, SF-476, SF-477, SF-478, SF-479, SF-480, SF-481, SF-482, SF-483, SF-484, SF-485, SF-486, SF-487, SF-488, SF-489, SF-490, SF-491, SF-492, SF-493, SF-494, SF-495, SF-496, SF-497, SF-498, SF-499, SF-500, SF-501, or SF-502.

30 7. The method according to claim 1, 2, or 5, wherein step (a) comprises isoelectric focussing followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

35 8. A method for screening, diagnosis or prognosis of Schizophrenia in a subject, for determining the stage or severity of Schizophrenia in a subject, for identifying a subject at risk of developing Schizophrenia, or for monitoring the effect of therapy administered to a

subject having Schizophrenia, said method comprising detecting, in a sample of cerebrospinal fluid from the subject, at least one of the following Schizophrenia-Associated Protein Isoforms (SPIs): SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-15, SPI-16, SPI-17, SPI-18, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-40, SPI-41, SPI-42, SPI-43, SPI-44, SPI-54, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-95, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-106, SPI-107, SPI-108, SPI-109, SPI-110, SPI-111, SPI-112, SPI-113, SPI-114, SPI-115, SPI-116, SPI-117, SPI-118, SPI-119, SPI-120, SPI-121, SPI-122, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-141, SPI-142, SPI-143, SPI-151, SPI-152, SPI-154, SPI-155, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-189, SPI-190, SPI-193, SPI-195, SPI-200, SPI-205, SPI-206, SPI-211, SPI-213, SPI-214, SPI-231, SPI-232, SPI-233, SPI-234, SPI-235, SPI-236, SPI-237, SPI-238, SPI-239, SPI-240, SPI-241, SPI-242, SPI-243, SPI-244, SPI-245, SPI-246, SPI-247, SPI-248, SPI-249, SPI-250, SPI-251, SPI-252, SPI-253, SPI-254, SPI-255, SPI-256, SPI-257, SPI-258, SPI-259, SPI-261, SPI-262, SPI-263, SPI-264, SPI-265, SPI-266, SPI-267, SPI-268, SPI-269, SPI-270, SPI-271, SPI-272, SPI-273, SPI-274, SPI-275, SPI-276, SPI-277, SPI-278, SPI-279, SPI-280, SPI-281, SPI-282, SPI-283, SPI-284, SPI-285, SPI-286, SPI-287, SPI-288, SPI-289, SPI-290, SPI-291, SPI-292, SPI-293, SPI-294, SPI-295, SPI-296, SPI-297, SPI-298, SPI-299, SPI-300, SPI-301, SPI-302, SPI-303, SPI-304, SPI-312, SPI-321, SPI-322, SPI-323, SPI-324, SPI-325, SPI-326, SPI-327, SPI-328, SPI-329, SPI-331, SPI-332, SPI-334, SPI-335, SPI-336, SPI-337, SPI-338, SPI-339, SPI-340, SPI-341, SPI-342, SPI-343, SPI-344, SPI-345, SPI-346, SPI-348, SPI-349, SPI-352, SPI-353, SPI-354, SPI-355, SPI-357, SPI-359, SPI-360, SPI-361, SPI-362, SPI-363, SPI-365, SPI-367, SPI-369, SPI-370, SPI-372, SPI-375, SPI-376, SPI-379, SPI-381, SPI-382, SPI-383, SPI-384, SPI-385, SPI-387, SPI-388, SPI-389, SPI-390, SPI-391, SPI-392, SPI-393, SPI-394, SPI-397, SPI-398, SPI-399, SPI-400, SPI-401, SPI-402, SPI-403, SPI-404, SPI-405, SPI-406, SPI-407, SPI-408, SPI-409, SPI-410, SPI-411, SPI-412, SPI-413, SPI-414, SPI-415, SPI-416, SPI-417, SPI-418, SPI-419, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, SPI-427, SPI-428, SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, or SPI-467.

9. The method according to claim 8, wherein the step of detecting comprises testing at least one aliquot of the sample, said step of testing comprising:

35 (a) contacting the aliquot with an antibody that is immunospecific for a preselected SPI; and

(b) measuring any binding that has occurred between the antibody and at least one species in the aliquot.

10. The method according to claim 9, wherein the antibody is a monoclonal antibody.

11. The method according to claim 9, wherein the step of detecting comprises testing a plurality of aliquots with a plurality of antibodies for detection of a plurality of preselected SPIs.

12. The method according to claim 11, wherein the antibodies are monoclonal antibodies.

13. A preparation comprising one of the following isolated Schizophrenia-Associated Protein Isoforms (SPIs): SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-15, SPI-16, SPI-17, SPI-18, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-41, SPI-42, SPI-43, SPI-44, SPI-54, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-71, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-84, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-95, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-107, SPI-113, SPI-114, SPI-115, SPI-116, SPI-118, SPI-121, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-139, SPI-141, SPI-142, SPI-143, SPI-151, SPI-152, SPI-154, SPI-155, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-186, SPI-187, SPI-188, SPI-189, SPI-190, SPI-191, SPI-192, SPI-193, SPI-195, SPI-201, SPI-205, SPI-206, SPI-211, SPI-213, SPI-214, SPI-231, SPI-232, SPI-234, SPI-235, SPI-236, SPI-237, SPI-238, SPI-239, SPI-240, SPI-241, SPI-242, SPI-243, SPI-244, SPI-245, SPI-246, SPI-247, SPI-248, SPI-249, SPI-250, SPI-251, SPI-252, SPI-253, SPI-254, SPI-255, SPI-257, SPI-258, SPI-259, SPI-261, SPI-262, SPI-263, SPI-264, SPI-265, SPI-266, SPI-267, SPI-268, SPI-269, SPI-270, SPI-271, SPI-272, SPI-273, SPI-274, SPI-275, SPI-278, SPI-281, SPI-285, SPI-289, SPI-290, SPI-296, SPI-302, SPI-303, SPI-306, SPI-312, SPI-321, SPI-322, SPI-323, SPI-325, SPI-326, SPI-327, SPI-328, SPI-329, SPI-331, SPI-332, SPI-334, SPI-335, SPI-336, SPI-337, SPI-338, SPI-339, SPI-340, SPI-341, SPI-342, SPI-343, SPI-344, SPI-345, SPI-346, SPI-347, SPI-349, SPI-351, SPI-352, SPI-353, SPI-354, SPI-355, SPI-357, SPI-359, SPI-360, SPI-362, SPI-363, SPI-365, SPI-367, SPI-369, SPI-370, SPI-372, SPI-375, SPI-376, SPI-379, SPI-381, SPI-382, SPI-383, SPI-384, SPI-385, SPI-387, SPI-388, SPI-389, SPI-390, SPI-391, SPI-392, SPI-393, SPI-394, SPI-396, SPI-397, SPI-398, SPI-399, SPI-400, SPI-401, SPI-402, SPI-403, SPI-404, SPI-405, SPI-406, SPI-407, SPI-408, SPI-409, SPI-410, SPI-411, SPI-412, SPI-413, SPI-414, SPI-415, SPI-416, SPI-417, SPI-418, SPI-419, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, SPI-427, SPI-428, SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, or SPI-467.

35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 109 110 111 112 113 114 115 116 117 118 119 119 120 121 122 123 124 125 126 127 128 129 129 130 131 132 133 134 135 136 137 138 139 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 179 180 181 182 183 184 185 186 187 188 189 189 190 191 192 193 194 195 196 197 198 199 199 200 201 202 203 204 205 206 207 208 209 209 210 211 212 213 214 215 216 217 218 219 219 220 221 222 223 224 225 226 227 228 229 229 230 231 232 233 234 235 236 237 238 239 239 240 241 242 243 244 245 246 247 248 249 249 250 251 252 253 254 255 256 257 258 259 259 260 261 262 263 264 265 266 267 268 269 269 270 271 272 273 274 275 276 277 278 279 279 280 281 282 283 284 285 286 287 288 289 289 290 291 292 293 294 295 296 297 298 299 299 300 301 302 303 304 305 306 307 308 309 309 310 311 312 313 314 315 316 317 318 319 319 320 321 322 323 324 325 326 327 328 329 329 330 331 332 333 334 335 336 337 338 339 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 369 370 371 372 373 374 375 376 377 378 379 379 380 381 382 383 384 385 386 387 388 389 389 390 391 392 393 394 395 396 397 398 399 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467.

- SPI-411, SPI-412, SPI-413, SPI-414, SPI-415, SPI-416, SPI-417, SPI-418, SPI-419, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, SPI-427, SPI-428, SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, or SPI-467.

14. A kit comprising the preparation of claim 13.

10 15. A kit comprising a plurality of distinct preparations of claim 14...

16. An antibody capable of immunospecific binding to one of the following Schizophrenia-Associated Protein Isoforms (SPIs): SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-15, SPI-16, SPI-17, SPI-18, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-41, SPI-42, SPI-43, SPI-44, SPI-54, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-95, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-107, SPI-113, SPI-114, SPI-115, SPI-118, SPI-122, SPI-123, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-139, SPI-141, SPI-142, SPI-143, SPI-151, SPI-154, SPI-155, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-189, SPI-190, SPI-193, SPI-195, SPI-200, SPI-205, SPI-206, SPI-211, SPI-213, SPI-214, SPI-231, SPI-232, SPI-233, SPI-234, SPI-235, SPI-236, SPI-237, SPI-238, SPI-239, SPI-240, SPI-241, SPI-242, SPI-243, SPI-244, SPI-245, SPI-246, SPI-247, SPI-248, SPI-249, SPI-250, SPI-251, SPI-252, SPI-253, SPI-254, SPI-255, SPI-257, SPI-258, SPI-259, SPI-261, SPI-262, SPI-263, SPI-264, SPI-265, SPI-266, SPI-267, SPI-268, SPI-269, SPI-270, SPI-273, SPI-274, SPI-275, SPI-278, SPI-281, SPI-285, SPI-289, SPI-290, SPI-296, SPI-302, SPI-303, SPI-306, SPI-312, SPI-321, SPI-322, SPI-323, SPI-324, SPI-325, SPI-326, SPI-327, SPI-328, SPI-329, SPI-331, SPI-332, SPI-334, SPI-335, SPI-336, SPI-337, SPI-338, SPI-339, SPI-340, SPI-341, SPI-342, SPI-343, SPI-344, SPI-345, SPI-346, SPI-348, SPI-349, SPI-352, SPI-353, SPI-354, SPI-355, SPI-357, SPI-359, SPI-360, SPI-361, SPI-362, SPI-363, SPI-365, SPI-367, SPI-369, SPI-370, SPI-372, SPI-375, SPI-379, SPI-381, SPI-382, SPI-383, SPI-384, SPI-385, SPI-387, SPI-388, SPI-389, SPI-390, SPI-391, SPI-392, SPI-393, SPI-394, SPI-397, SPI-398, SPI-399, SPI-400, SPI-401, SPI-402, SPI-403, SPI-404, SPI-405, SPI-406, SPI-407, SPI-408, SPI-409, SPI-410, SPI-411, SPI-412, SPI-413, SPI-414, SPI-415, SPI-416, SPI-417, SPI-418, SPI-419, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, SPI-427, SPI-428, SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, or SPI-467.

20 25. A method of treating or preventing Schizophrenia comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of a nucleic acid that inhibits the function of one or more of the following Schizophrenia-Associated Protein Isoforms (SPIs): SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-

- 25 25, SPI-16, SPI-17, SPI-18, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-41, SPI-42, SPI-43, SPI-44, SPI-54, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-95, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-107, SPI-113, SPI-114, SPI-115, SPI-118, SPI-122, SPI-123, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-139, SPI-141, SPI-142, SPI-143, SPI-151, SPI-152, SPI-154, SPI-155, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-189, SPI-190, SPI-193, SPI-195, SPI-200, SPI-205, SPI-206, SPI-211, SPI-214, SPI-213, SPI-214, SPI-223, SPI-224, SPI-225, SPI-226, SPI-227, SPI-228, SPI-229, SPI-230, SPI-240, SPI-241, SPI-242, SPI-243, SPI-244, SPI-245, SPI-246, SPI-247, SPI-248, SPI-249, SPI-250, SPI-251, SPI-252, SPI-253, SPI-254, SPI-255, SPI-256, SPI-257, SPI-258, SPI-259, SPI-260, SPI-261, SPI-262, SPI-263, SPI-264, SPI-265, SPI-266, SPI-267, SPI-268, SPI-269, SPI-270, SPI-273, SPI-274, SPI-275, SPI-278, SPI-281, SPI-285, SPI-289, SPI-290, SPI-296, SPI-302, SPI-303, SPI-306, SPI-312, SPI-321, SPI-322, SPI-323, SPI-324, SPI-325, SPI-326, SPI-327, SPI-328, SPI-329, SPI-331, SPI-332, SPI-334, SPI-335, SPI-336, SPI-337, SPI-338, SPI-339, SPI-340, SPI-341, SPI-342, SPI-343, SPI-344, SPI-345, SPI-346, SPI-348, SPI-349, SPI-352, SPI-353, SPI-354, SPI-355, SPI-357, SPI-359, SPI-360, SPI-361, SPI-362, SPI-363, SPI-365, SPI-367, SPI-369, SPI-370, SPI-372, SPI-375, SPI-379, SPI-381, SPI-382, SPI-383, SPI-384, SPI-385, SPI-387, SPI-388, SPI-389, SPI-390, SPI-391, SPI-392, SPI-393, SPI-394, SPI-397, SPI-398, SPI-399, SPI-400, SPI-401, SPI-402, SPI-403, SPI-404, SPI-405, SPI-406, SPI-407, SPI-408, SPI-409, SPI-410, SPI-411, SPI-412, SPI-413, SPI-414, SPI-415, SPI-416, SPI-417, SPI-418, SPI-419, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, SPI-427, SPI-428, SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, or SPI-467.

- PCT/GB01/00783
- SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, or SPI-467.

5 17. The antibody of claim 16, which is a monoclonal antibody.

10 18. The antibody of claim 16 or 17, which binds to the SPI with greater affinity than to another isoform of the SPI.

15 19. The antibody of claim 16, which binds to the SPI with greater affinity than to any other isoform of the SPI.

20 20. A kit comprising the antibody of claim 16.

21. A kit comprising a plurality of distinct antibodies of claim 16.

22. A pharmaceutical composition comprising a therapeutically effective amount of an antibody of claim 16 and a pharmaceutically acceptable carrier.

25 23. A pharmaceutical composition comprising a therapeutically effective amount of a fragment or derivative of an antibody of claim 16, said fragment or derivative containing the binding domain of the antibody; and a pharmaceutically acceptable carrier.

24. A method of treating or preventing Schizophrenia comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of a nucleic acid encoding one of the following Schizophrenia-Associated Protein Isoforms (SPIs): SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-15, SPI-16, SPI-17, SPI-18, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-41, SPI-42, SPI-43, SPI-44, SPI-54, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-94, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-107, SPI-113, SPI-114, SPI-118, SPI-122, SPI-123, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-139, SPI-141, SPI-142, SPI-143, SPI-151, SPI-152, SPI-154, SPI-155, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-189, SPI-190, SPI-193, SPI-195, SPI-200, SPI-205, SPI-206, SPI-211, SPI-214, SPI-215, SPI-216, SPI-217, SPI-218, SPI-219, SPI-220, SPI-221, SPI-222, SPI-223, SPI-224, SPI-225, SPI-226, SPI-227, SPI-228, SPI-229, SPI-230, SPI-231, SPI-232, SPI-233, SPI-234, SPI-235, SPI-236, SPI-237, SPI-238, SPI-239, SPI-240, SPI-241, SPI-242, SPI-243, SPI-244, SPI-245, SPI-246, SPI-247, SPI-248, SPI-249, SPI-250, SPI-251, SPI-252, SPI-253, SPI-254, SPI-255, SPI-256, SPI-257, SPI-258, SPI-259, SPI-261, SPI-262, SPI-263, SPI-264,

- SPI-265, SPI-266, SPI-267, SPI-268, SPI-269, SPI-270, SPI-273, SPI-274, SPI-275, SPI-278, SPI-281, SPI-285, SPI-289, SPI-290, SPI-296, SPI-302, SPI-303, SPI-306, SPI-312, SPI-321, SPI-322, SPI-323, SPI-324, SPI-325, SPI-326, SPI-327, SPI-328, SPI-329, SPI-331, SPI-332, SPI-334, SPI-335, SPI-336, SPI-337, SPI-338, SPI-339, SPI-340, SPI-341, SPI-342, SPI-343, SPI-344, SPI-345, SPI-346, SPI-348, SPI-349, SPI-352, SPI-353, SPI-354, SPI-355, SPI-357, SPI-359, SPI-360, SPI-361, SPI-362, SPI-363, SPI-365, SPI-367, SPI-369, SPI-370, SPI-373, SPI-376, SPI-377, SPI-378, SPI-379, SPI-381, SPI-382, SPI-383, SPI-384, SPI-385, SPI-387, SPI-388, SPI-389, SPI-390, SPI-391, SPI-392, SPI-393, SPI-394, SPI-397, SPI-398, SPI-399, SPI-400, SPI-401, SPI-402, SPI-403, SPI-404, SPI-405, SPI-406, SPI-407, SPI-408, SPI-409, SPI-410, SPI-411, SPI-412, SPI-413, SPI-414, SPI-415, SPI-416, SPI-417, SPI-418, SPI-419, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, SPI-427, SPI-428, SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, or SPI-467.

- 20 25. A method of treating or preventing Schizophrenia comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of a nucleic acid that inhibits the function of one or more of the following Schizophrenia-Associated Protein Isoforms (SPIs): SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-

- 5 25, SPI-16, SPI-17, SPI-18, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-41, SPI-42, SPI-43, SPI-44, SPI-54, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-95, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-107, SPI-113, SPI-114, SPI-118, SPI-122, SPI-123, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-139, SPI-141, SPI-142, SPI-143, SPI-151, SPI-152, SPI-154, SPI-155, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-189, SPI-190, SPI-193, SPI-195, SPI-200, SPI-205, SPI-206, SPI-211, SPI-214, SPI-215, SPI-216, SPI-217, SPI-218, SPI-219, SPI-220, SPI-221, SPI-222, SPI-223, SPI-224, SPI-225, SPI-226, SPI-227, SPI-228, SPI-229, SPI-230, SPI-231, SPI-232, SPI-233, SPI-234, SPI-235, SPI-236, SPI-237, SPI-238, SPI-239, SPI-240, SPI-241, SPI-242, SPI-243, SPI-244, SPI-245, SPI-246, SPI-247, SPI-248, SPI-249, SPI-250, SPI-251, SPI-252, SPI-253, SPI-254, SPI-255, SPI-256, SPI-257, SPI-258, SPI-259, SPI-261, SPI-262, SPI-263, SPI-264,

- 10 27. A method of screening for agents that interact with an SPI, an SPI fragment, or an SPI-related polypeptide, said method comprising:  
 (a) contacting an SPI, a biologically active portion of an SPI, or an SPI-related polypeptide with a candidate agent; and  
 (b) determining whether or not the candidate agent interacts with the SPI, the SPI fragment, or the SPI-related polypeptide.
- 15 28. The method of claim 27, wherein the SPI, the SPI fragment, or the SPI-related polypeptide is expressed by cells.
- 20 29. The method of claim 27, wherein the cells express a recombinant SPI, a recombinant SPI fragment, or a recombinant SPI-related polypeptide.
- 25 30. A method of screening for agents that modulate the expression or activity of an SPI or an SPI-related polypeptide comprising:  
 (a) contacting a first population of cells expressing an SPI or an SPI-related polypeptide with a candidate agent;

(b) contacting a second population of cells expressing said SPI or said SPI-related polypeptide with a control agent; and

(c) comparing the level of said SPI or said SPI-related polypeptide or mRNA encoding said SPI or said SPI-related polypeptide in the first and second populations of cells, or comparing the level of induction of a cellular second messenger in the first and second populations of cells.

31. The method of claim 30, wherein the level of said SPI or said SPI-related polypeptide, mRNA encoding said SPI or said SPI-related polypeptide, or said cellular second messenger is greater in the first population of cells than in the second population of cells.

32. The method of claim 30, wherein the level of said SPI or said SPI-related polypeptide, mRNA encoding said SPI or said SPI-related polypeptide, or said cellular second messenger is less in the first population of cells than in the second population of cells.

33. A method of screening for or identifying agents that modulate the expression or activity of an SPI or an SPI-related polypeptide comprising:

- (a) administering a candidate agent to a first mammal or group of mammals;
- (b) administering a control agent to a second mammal or group of mammals;

20 and

(c) comparing the level of expression of the SPI or the SPI-related polypeptide or of mRNA encoding the SPI or the SPI-related polypeptide in the first and second groups, or comparing the level of induction of a cellular second messenger in the first and second groups.

25 34. The method of claim 33, wherein the mammals are animal models for Schizophrenia.

35. The method of claim 33 or 34, wherein the level of expression of said SPI or said SPI-related polypeptide, mRNA encoding said SPI or said SPI-related polypeptide, or of said cellular second messenger is greater in the first group than in the second group.

36. The method of claim 33 or 34, wherein the level of expression of said SPI or said SPI-related polypeptide, mRNA encoding said SPI or said SPI-related polypeptide, or of said cellular second messenger is less than in the first group than in the second group.

37. The method of claim 33, wherein the levels of said SPI or said SPI-related polypeptide, mRNA encoding said SPI or said SPI-related polypeptide, or of said cellular second messenger in the first and second groups are further compared to the level of said SPI or said SPI-related polypeptide or said mRNA encoding said SPI or said SPI-related polypeptide in normal control mammals.

38. The method of claim 33, wherein administration of said candidate agent modulates the level of said SPI or said SPI-related polypeptide, or said mRNA encoding said SPI or said SPI-related polypeptide, or said cellular second messenger in the first group towards the levels of said SPI or said SPI-related polypeptide or said mRNA or said cellular second messenger in the second group.

39. The method of claim 33, wherein said mammals are human subjects having Schizophrenia.

40. A method of screening for or identifying agents that interact with an SPI or an SPI-related polypeptide, comprising (a) contacting a candidate agent with the SPI or the SPI-related polypeptide, and (b) detecting binding, if any, between the agent and the SPI or the SPI-related polypeptide.

41. A method of screening for or identifying agents that modulate the activity of an SPI or an SPI-related polypeptide, comprising: (a) in a first aliquot, contacting a candidate agent with the SPI or the SPI-related polypeptide, and (b) comparing the activity of the SPI or the SPI-related polypeptide in the first aliquot after addition of the candidate agent with the activity of the SPI or the SPI-related polypeptide in a control aliquot, or with a previously determined reference range.

42. The method according to claim 40 or 41, wherein the SPI or the SPI-related polypeptide is recombinant protein.

43. The method according to claim 40 or 41, wherein the SPI or the SPI-related polypeptide is immobilized on a solid phase.

44. A method for screening, diagnosis or prognosis of Schizophrenia in a subject or for monitoring the effect of an anti-Schizophrenia drug or therapy administered to a subject, comprising:

(a) contacting at least one oligonucleotide probe comprising 10 or more consecutive nucleotides complementary to a nucleotide sequence encoding an SPI chosen from SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-15, SPI-16, SPI-17, SPI-18, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-41, SPI-42, SPI-43, SPI-44, SPI-54, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-95, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-107, SPI-113, SPI-114, SPI-115, SPI-118, SPI-122, SPI-123, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-139, SPI-141, SPI-142, SPI-143, SPI-151, SPI-152, SPI-154, SPI-155, SPI-156, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-189, SPI-190, SPI-193, SPI-195, SPI-200, SPI-205, SPI-206, SPI-211, SPI-213, SPI-214, SPI-231, SPI-232, SPI-233, SPI-234, SPI-235, SPI-236, SPI-237, SPI-238, SPI-239, SPI-240, SPI-241, SPI-242, SPI-243, SPI-244, SPI-245, SPI-246, SPI-247, SPI-248, SPI-249, SPI-250, SPI-251, SPI-252, SPI-253, SPI-254, SPI-255, SPI-257, SPI-258, SPI-259, SPI-261, SPI-262, SPI-263, SPI-264, SPI-265, SPI-266, SPI-267, SPI-268, SPI-269, SPI-270, SPI-273, SPI-274, SPI-275, SPI-278, SPI-281, SPI-285, SPI-289, SPI-290, SPI-296, SPI-302, SPI-303, SPI-306, SPI-312, SPI-321, SPI-322, SPI-323, SPI-324, SPI-325, SPI-326, SPI-327, SPI-328, SPI-331, SPI-332, SPI-334, SPI-335, SPI-336, SPI-337, SPI-338, SPI-339, SPI-340, SPI-341, SPI-342, SPI-343, SPI-344, SPI-345, SPI-346, SPI-348, SPI-349, SPI-352, SPI-353, SPI-354, SPI-355, SPI-357, SPI-359, SPI-360, SPI-361, SPI-362, SPI-363, SPI-365, SPI-367, SPI-369, SPI-370, SPI-372, SPI-375, SPI-376, SPI-379, SPI-381, SPI-382, SPI-383, SPI-384, SPI-385, SPI-387, SPI-388, SPI-389, SPI-390, SPI-391, SPI-392, SPI-393, SPI-394, SPI-395, SPI-396, SPI-397, SPI-398, SPI-399, SPI-400, SPI-401, SPI-402, SPI-403, SPI-404, SPI-405, SPI-406, SPI-407, SPI-408, SPI-409, SPI-410, SPI-411, SPI-412, SPI-413, SPI-414, SPI-415, SPI-416, SPI-417, SPI-418, SPI-419, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, SPI-427, SPI-428, SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, or SPI-467 with mRNA obtained from a biological sample from the subject or with cDNA copied from the RNA wherein said contacting occurs under conditions that permit hybridization of the probe to the nucleotide sequence if present;

35 and

(c) comparing the hybridization, if any, detected in step (b) with the hybridization detected in a control sample, or with a previously determined reference range.

45. The method of claim 44, wherein step (a) comprises contacting a plurality of oligonucleotide probes comprising 10 or more consecutive nucleotides complementary to a nucleotide sequence encoding an SPI chosen from SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-15, SPI-16, SPI-17, SPI-18, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-41, SPI-42, SPI-43, SPI-44, SPI-54, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-95, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-107, SPI-113, SPI-114, SPI-115, SPI-118, SPI-122, SPI-123, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-139, SPI-141, SPI-142, SPI-143, SPI-151, SPI-152, SPI-154, SPI-155, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-189, SPI-190, SPI-193, SPI-195, SPI-200, SPI-205, SPI-206, SPI-211, SPI-213, SPI-214, SPI-217, SPI-218, SPI-219, SPI-220, SPI-221, SPI-222, SPI-223, SPI-224, SPI-225, SPI-226, SPI-227, SPI-228, SPI-229, SPI-230, SPI-231, SPI-232, SPI-233, SPI-234, SPI-235, SPI-236, SPI-237, SPI-238, SPI-239, SPI-240, SPI-241, SPI-242, SPI-243, SPI-244, SPI-245, SPI-246, SPI-247, SPI-248, SPI-249, SPI-250, SPI-251, SPI-252, SPI-253, SPI-254, SPI-255, SPI-257, SPI-258, SPI-259, SPI-261, SPI-262, SPI-263, SPI-264, SPI-265, SPI-266, SPI-267, SPI-268, SPI-269, SPI-270, SPI-273, SPI-274, SPI-275, SPI-278, SPI-281, SPI-285, SPI-289, SPI-290, SPI-291, SPI-302, SPI-303, SPI-306, SPI-312, SPI-312, SPI-321, SPI-322, SPI-323, SPI-324, SPI-325, SPI-326, SPI-327, SPI-328, SPI-329, SPI-331, SPI-332, SPI-334, SPI-335, SPI-336, SPI-337, SPI-338, SPI-339, SPI-340, SPI-341, SPI-342, SPI-343, SPI-344, SPI-345, SPI-346, SPI-348, SPI-349, SPI-352, SPI-353, SPI-354, SPI-355, SPI-357, SPI-359, SPI-360, SPI-361, SPI-362, SPI-363, SPI-365, SPI-367, SPI-369, SPI-370, SPI-372, SPI-375, SPI-376, SPI-379, SPI-381, SPI-382, SPI-383, SPI-384, SPI-385, SPI-387, SPI-388, SPI-389, SPI-390, SPI-391, SPI-392, SPI-393, SPI-394, SPI-395, SPI-396, SPI-397, SPI-398, SPI-399, SPI-400, SPI-401, SPI-402, SPI-403, SPI-404, SPI-405, SPI-406, SPI-407, SPI-408, SPI-409, SPI-410, SPI-411, SPI-412, SPI-413, SPI-414, SPI-415, SPI-416, SPI-417, SPI-418, SPI-419, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, SPI-427, SPI-428, SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, or SPI-467 with mRNA obtained from a biological sample from the subject or with cDNA copied from the RNA wherein said contacting occurs under conditions that permit hybridization of the probe to the nucleotide sequence if present;

25 46. The method of claim 44, wherein step (a) comprises contacting a plurality of oligonucleotide probes comprising 10 or more consecutive nucleotides complementary to a nucleotide sequence encoding an SPI chosen from SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-11, SPI-13, SPI-15, SPI-16, SPI-17, SPI-18, SPI-19, SPI-20, SPI-21, SPI-23, SPI-24, SPI-25, SPI-26, SPI-28, SPI-29, SPI-30, SPI-32, SPI-33, SPI-34, SPI-35, SPI-36, SPI-38, SPI-39, SPI-41, SPI-42, SPI-43, SPI-44, SPI-54, SPI-56, SPI-57, SPI-58, SPI-59, SPI-60, SPI-62, SPI-63, SPI-65, SPI-67, SPI-69, SPI-73, SPI-74, SPI-75, SPI-76, SPI-77, SPI-78, SPI-80, SPI-81, SPI-82, SPI-83, SPI-85, SPI-87, SPI-88, SPI-91, SPI-92, SPI-93, SPI-95, SPI-96, SPI-97, SPI-99, SPI-100, SPI-101, SPI-105, SPI-107, SPI-113, SPI-114, SPI-115, SPI-118, SPI-122, SPI-123, SPI-124, SPI-127, SPI-129, SPI-130, SPI-132, SPI-133, SPI-138, SPI-139, SPI-141, SPI-142, SPI-143, SPI-151, SPI-152, SPI-154, SPI-155, SPI-164, SPI-166, SPI-167, SPI-169, SPI-170, SPI-175, SPI-176, SPI-179, SPI-181, SPI-182, SPI-183, SPI-184, SPI-185, SPI-189, SPI-190, SPI-193, SPI-195, SPI-200, SPI-205, SPI-206, SPI-211, SPI-213, SPI-214, SPI-217, SPI-218, SPI-219, SPI-220, SPI-221, SPI-222, SPI-223, SPI-224, SPI-225, SPI-226, SPI-227, SPI-228, SPI-229, SPI-230, SPI-312, SPI-312, SPI-321, SPI-322, SPI-323, SPI-324, SPI-325, SPI-326, SPI-327, SPI-328, SPI-329, SPI-331, SPI-332, SPI-334, SPI-335, SPI-336, SPI-337, SPI-338, SPI-339, SPI-340, SPI-341, SPI-342, SPI-343, SPI-344, SPI-345, SPI-346, SPI-348, SPI-349, SPI-352, SPI-353, SPI-354, SPI-355, SPI-357, SPI-359, SPI-360, SPI-361, SPI-362, SPI-363, SPI-365, SPI-367, SPI-370, SPI-372, SPI-375, SPI-376, SPI-379, SPI-381, SPI-382, SPI-383, SPI-384, SPI-385, SPI-387, SPI-388, SPI-389, SPI-390, SPI-391, SPI-392, SPI-393, SPI-394, SPI-395, SPI-396, SPI-397, SPI-398, SPI-399, SPI-400, SPI-401, SPI-402, SPI-403, SPI-404, SPI-405, SPI-406, SPI-407, SPI-408, SPI-409, SPI-410, SPI-411, SPI-412, SPI-413, SPI-414, SPI-415, SPI-416, SPI-417, SPI-418, SPI-419, SPI-420, SPI-421, SPI-422, SPI-423, SPI-424, SPI-425, SPI-426, SPI-427, SPI-428, SPI-429, SPI-430, SPI-431, SPI-432, SPI-433, SPI-434, SPI-435, SPI-436, SPI-437, SPI-438, SPI-439, SPI-440, SPI-441, SPI-442, SPI-443, SPI-444, SPI-445, SPI-446, SPI-447, SPI-448, SPI-449, SPI-450, SPI-451, SPI-452, SPI-453, SPI-454, SPI-455, SPI-456, SPI-457, SPI-458, SPI-459, SPI-460, SPI-461, SPI-462, SPI-463, SPI-464, SPI-465, SPI-466, or SPI-467 with mRNA obtained from a biological sample from the subject or with cDNA copied from the RNA wherein said contacting occurs under conditions that permit hybridization of the probe to the nucleotide sequence if present;

35 and

(c) comparing the hybridization, if any, detected in step (b) with the hybridization detected in a control sample, or with a previously determined reference range.

46. The method of claim 44, wherein step (a) includes the step of hybridizing the nucleotide sequence to a DNA array, wherein one or more members of the array are the probes complementary to a plurality of nucleotide sequences encoding distinct SPIs.

5 47. A method of screening for agents effective for the treatment of Schizophrenia comprising:

- (a) contacting NP-1 with a first population of cells expressing a receptor for NP-1 (hNPR) in the presence of a candidate agent;
- (b) contacting NP-1 with a second population of cells expressing said receptor (hNPR) in the presence of a control agent;
- 10 (c) comparing the binding of said NP-1 to the first and second populations of cells, or comparing the level of induction of a cellular second messenger in the first and second populations of cells, or comparing the level of a NP-1 mediated activity in the first and second population of cells; and
- 15 (d) testing for the ability of agents able to modulate the activity of NP-1 to decrease clinical features of Schizophrenia in a Schizophrenia disease model system.

48. The method of claim 47 wherein the NP-1 or the receptor, or both, are isolated and recombinantly produced.

20 49. The method of claim 47, wherein the cells are glial cells and the NP-1 mediated activity is causing the glial cells to be susceptible to neurotoxin activity.

25 50. The method of claim 49, wherein the neurotoxin is taipoxin.

51. The method of claim 47, wherein the candidate agent comprises at least 10 consecutive amino acids of NP-1.

30 52. A method of screening for agents that modulate the binding of NP-1 to a binding partner comprising:

- (a) contacting NP-1 with the NP-1 binding partner in the presence of a candidate agent;
- (b) contacting NP-1 with a NP-1 binding partner in the presence of a control agent; and
- 35 (c) comparing the binding of said NP-1 to the binding partner in step (a) to the binding of said NP-1 to the binding partner in step (b).

53. The method of claim 51 wherein the binding partner is an isolated NP-1 receptor (hNPR).

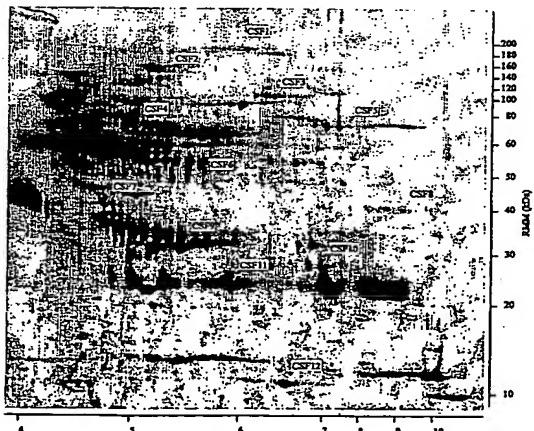


FIGURE 1

R L T L K F L A V L L A A G M L A F I G  
A V I C I P A S V P L A A S P A R A L P  
G G A D N A S V A S G A A A S P G P O R  
S L S A L H G A G G S A G P P A L P G A  
P A A S A H P L P P G P L F S R F L C T  
P L A A A C P S G A Q Q G D A A G A A P  
G E R E E L L L L Q S T A E Q L R Q T A  
L Q Q E A R I R A D Q D T I R E L T G K  
L G R C E S G L P R G L Q G A G P R R D  
T H A D G P W D S P A L I L E L E D A V  
R A L R D R I D R L E E L P A R V N L S  
A A P A P V S A V P T G L H S K M D Q L  
E G Q L L A Q V L A L E K E R V A L S H  
S S R R Q R Q E V E K E L D V E S Q G N H R V  
A E L E H G S S A Y S P P D A F K I S I  
P I R N Y M Y A R V R K A L P E L Y A  
F T A C M W L R S R S S G T G Q G T P F  
S Y S V P G Q A N E I V L L E A G H E P  
M E L L I N D K V A Q L P L S L K D H G  
W H B I C I A W T T R D G L W S A Y Q D  
G E L Q G S G E N L A A W H P I K P H G  
I L D E L E G Q E Q D T L G G R F D A T Q A  
F V G D I A Q F N L W D H A L T P A Q V  
L G I A N C T A P L L G N V L P N E D K  
L V S A F G G A T K A A F D V C K G R A  
K A.

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**FIGURE 2B**

CGGCTCAAGCTGAACTCTGGCCGTGCTGGCCGCCGGCATGCTGGCCTTCCTCGGT  
GGCTCACTTCATCTCATCGGCCACGGCCCTGGCCGGCGACGCCCGCCGGCGCTTCGG  
GGGCCGCGCAGCACATGGCTCTGGCTCGTCCGGGCCGCCGGCCGGCGCTGGCCAGCGG  
AGCTTGACGGCGCTCAAGCGGCCGGGGCGGTGACGGCGGCCGGCCGGCGCTGGCCAGCGG  
CCCGCGGCCAGCGCGACCCGCTGGCCCGCCGGGCCCTGTCTAGCGCTTCTCTGACAGG  
CCGGCTGCTGCTGGCTGGCCCTGGGGGCCACAGGGGACCCGGCCGGCGCTGGCC  
GGCAGCGCGAAGACTGCTGCTGCGAGACGGCGACCCGGCGACGACTGGCCGAAGCGG  
CTGACAGAACGGCGCGCATCGGCCGCCAACAGGACCATCTGAGCTCTACCGGAAG  
CTGGCCGCTGCAAGCGGCCCTGGCCGGCGCTCGAAGGGCGGGGGCCCGGCCGGCGAC  
ACCATGGCGACGGGGCTGGAGCTCGCTGCGCTCATCTGGAGCTGGNAGCGCCGCTG  
CGGCCCTGCGAGCGGCATCGGCCGCTGGAGGAGCTTCAGGGCTGTGAGCTTC  
GCTGGCCAGCCCCAGTCTCTGCTGTCAGGCCGGCTACACTTCAGATGGACCTGG  
GAGGGCGCTGCTGGCCAGTGTGGCACTGGAGGAGGGCTGGCCCTGGCGAC  
AGCAGCGCCGGCGAGGCCAGGAGCTGGAGAAGGACTGGAGCTCTGGCCCTCGAGGGCTGTG  
GCTGAGCTGGAGACGGCTCTCGACCTACAGTCTTCAGATGGCTCTGAGATGAGCATC  
CCCTACCTGTAACACTACATGTCAGCCGGCGCGAGCTGCTGGCCAGCTTATGAG  
TTACCGCGCTGATGGCTGGCGCTCAGGTTCCAAGGCCGCAAGGGCCAGCTTAC  
TCTCCACTCAGTGGCCGGCGAGGCCAACGAGATTGTACTCTGAGGGCGGCCATGAGCCC  
ATGGAGCTCTGTAACAGCAAGGGTGGCCCACTGCGGCCCTGAGCTGAGAACATGGC  
TGGCCACACATGTCATGGCTGGCGCAACRAGGAGTGGCTCTGGCTCTGGAC  
GGGGCTGGCGACGGGGCTGGCGRAAGCTGGCTGGCCGGGGCTGGCTCTGGAC  
ATCTTAACTCTGGGGCAAGGAGCAGGATACCCCTGGTGGGGCTGGATGCGCCACAGGGC  
TTTGTGGTGAACATGTCGGCCAGTTAACCTGTTGGGACACGCCCTGACACCAGGGCAGGTC  
CTGGGGCTGGCCACACTGCACTGGCCCACTGCGCAACGGCTCTGGGAGACAAAG  
TTGGGGAGGGCTTGGGGTCAACAAAGGCTGCTCTGGATGTCGCAAGGGAGGGCC  
AAGGATGAGGGGCC

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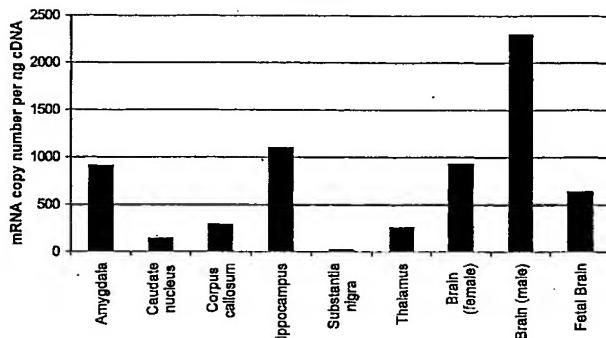
**FIGURE 4A**

M A S L L A V L L L L E R G M F S  
P S P P P A L L E K V F Q Y I D L H Q D E  
F V Q T L K H W V A I R S D S V Q P V P R  
F R Q G E L F X N M A V A R D T L Q R L G A  
R V A S V D M G P Q Q L P D G Q S L P I P  
P V I L A B L G S D P T K G T V C F Y G H  
L D V Q P A D R G D G W L T D P Y V L T E  
V D G K L Y G R G A T D N K G P V L A W I  
N A V S A F R A L E Q D L P V N I K P I I  
E G M E R A G S V A L E E L V B K E K D R  
F F S G V D Y I V I S D N L W . I S Q R K P  
A I T Y G T R G N S Y F M V V E V C R D Q  
D F H F S G T F G G I L H E P M A D L V A L  
L G S L W D S S G H I L V P G I Y D E B V V  
P L T E E E I N T Y K A I H L D H E B Y N  
N S S ' R V E K F L F D T K E R I L M H L W  
R Y P S L S I H G I E G A F D E P G T K T  
V I P G R V I G K G F S I R L V P H M N V S  
A V E K Q V T R H L E D F V F S K R N S S N  
K M V V S M T L G L H F W I A N I D D T Q  
Y L A A K R A I R T V F G T E P D M I R D  
G S T I F I A K M F Q R I V R H K S V V L I  
P L G A V V D D G E H S Q N E K I R N W N T  
I E G T K L F A A F P L E M A Q I H

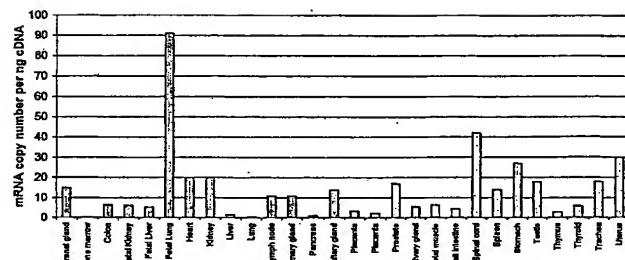
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**FIGURE 3**



## **Body tissues**



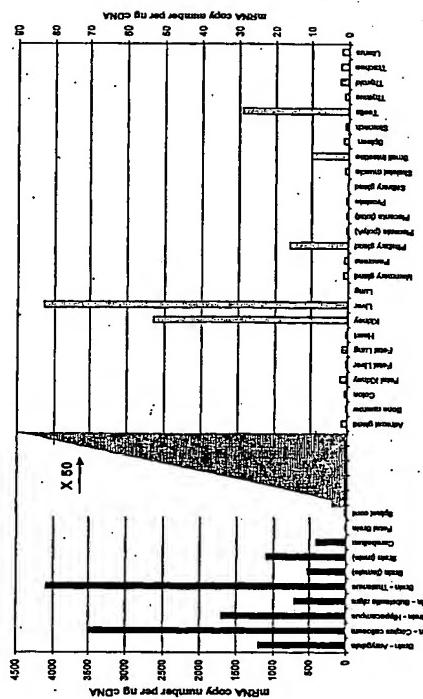
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**FIGURE 4B**

GNNNNNNNAGNGNTNTRNNNAAATGCGNCTTGTGANCATGGCTCGCTCTTGCTGGCTGTGCTGCT  
GTGCTGCTGCTGCTGCTGAGCCTGGCGATCTGTCTCTCCTACCCCTCCCCCCCCGGCGCTCTTAGAGA  
AGTCCTTCTCAGATCATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT  
CATGGAGGAGCAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT  
CGTGGCTTCGGCAACGGCTGCTGAGGGCTCTGGGGGCGCTGTGGCTCTGGGCTATGGCTCTGGC  
CGAGCTGCCGAGTGTCTGAGCTTCTCCGCTACCTCCGGCTACCTGGCTGAGGGAGCTGGCTCTGGC  
TCCCCAGAAGGACCCGGCTGTCTCTAGGGCCACTTGGACCTGGCTGAGGGAGCTTCTAGCTGAGGG  
TGGGTTGGCTCAGGGCCCTCTATGTCAGGGGGAGTGGACGGGAACTTCTAGCTGAGGGAC  
GAGGAGCAACAAAGGCGCTCTGTCTGGCTTGGATCAATGCTGTAGGGCCCTTCAGAGGCCCTTGG  
GCRAAGATCTTCCTGTGAAATTAATTCATCATGAGGGCTGAGGAGCTGGCTCTGGCTCTGGCT  
CTCTGGAGGAATTCTGGAAAAAGAAAAGGAGCCTTCTCTGGTGTGGACTACATTGTAAT  
TTCAAGATAACCTCTGGAACTGCCAAAGGAGGCCAGCACTTCTATGSAACCCCCGGGAAACNG  
CTACTCTATGGTGGAGGTTGGAAATGGAGGAGCAAGGATTCTTCACTGGAACTTCTGGTGGCAT  
CTCTCTGAGAACCTATGGCTGATCTGGTGTCTCTGGTGTGGAGTACTCTGGTGTCA  
TATCTGGTGTCTGGAACTATCTGATGAGGGTGTCTCTGGTGTGGAGTACTCTGGTGTCA  
CAAAGGCACTCCATCTAGACCTAGAAGAACTGGGAATAGCAGCAGCGGGTTGAGAAATTCTGT  
CGATACTAAAGGAGGAGATTCTAATGCACTCTGGAGGTACCCATCTTCTTCTATTCTGGGAT  
CGAGGGGGGGCTTGTGATGGCTGGAACTAAANAACTGATCTGGCTGGAGTTAGGAAAATT  
TCAATCTGGTGTCTGGTGTCTGGTGTGGGGAAATGAGGATCAAGCACGACATCT  
TGGAGATGTTCTTCTTCCAAAAGAATGTTCTAACAGATGTTGTCTTCTGATGACTCTGGAGT  
ACACCCGGTGGAGTGTGAAATTTGATGACACCCAGATCTGGAGCTGGAGCTGGAGCTGGAG  
AGTGTGTTGGAGACGAGACAGAGATATGATCTGGGGATGGATCTCCACCACTTGTGCAAAATGTT  
CCAGGAGAGTGTGCTCCAGAGGAGCTGGTGTCAATTCTGGGGAGCTGGTGTGAGTGGAGACA  
TTGGAGAGATGAGAAAATCTAACAGGTGGAACTCATAGAGGGAACAAATTATTTCTGGCTCT  
TTCTCTGGAGTGGGGCAAGATCTCATTAATCAGAGACTCTCTACTCTGATCTGATCTGAGCTGA  
CAGAACTTACCTTC

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FIGURE 5.



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ବୁଦ୍ଧିମତ୍ତା ପରିଚୟ ଓ ନିର୍ଣ୍ଣାଯକ ମାର୍ଗ

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ଲାଗୁ କରିବାକୁ ପାଇଁ ଆମେ ଏହାର